THE DUBININ EFFECT AND THE Y CHROMOSOME[1]

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DUBININ and SIDOROV (1934) found that certain translocations of the fourth chromosome in *Drosophila melanogaster* are associated with a weakened dominance of the wild type allele of the cubitus interruptus gene, ci^+, when they are tested against the *ci* mutant. Previously, it had been established that some genes, notably white and brown (MULLER 1930; GLASS 1933) normally located in euchromatin and brought into heterochromatin through a chromosomal rearrangement, also showed a weakened dominance of their wild type allele. In these latter cases, the action of the gene appeared to fluctuate in its expression from cell to cell or from one group of cells to another in the fly so that a somatic mosacism resulted and the term variegated-type position effect was applied to this class of phenomena (see Review by LEWIS 1950). The question as to whether the **R(ci$^+$)/ci** phenotype is variegated, where *R(ci$^+$)* symbolizes the rearranged chromosome, necessarily remains in abeyance since the *ci* mutant is variable in expression and since the character is limited to breaks in a single wing vein. KHVOSTOVA (1939) showed in a comprehensive cytological analysis that the ci^+ position effect is almost invariably associated with a euchromatic-heterochromatic type of rearrangement which involves a break in the proximal heterochromatin of chromosome four, close to the region of the *ci* gene.

The "Dubinin effect," a term often applied to position effects at the *ci* locus, differs from most V-type position effects in that *R(ci$^+$)* in the homozygous condition, or when present in one dose either as the hemizygote or when tested over a deficiency is wild type, whereas most *R(+)* when viable in the homozygote or hemizygote or when tested over a deficiency exhibit a variegated mutant phenotype. DUBININ and SIDOROV (1934) found, however, that *R(h$^+$)*, a euchromatic locus in 3L which shows a V-type position effect in combination with its mutant allele, hairy, although lethal in this case as a homozygote, is wild type when tested against a deficiency for the hairy locus. Another point of resemblance between *R(ci$^+$)* and *R(h$^+$)* is that the criterion for phenotypic variegation, i.e., somatic mosacism for the expression of both the mutant and the wild type alleles, cannot be satisfactorily applied in either case.

An important criterion for the recognition of a V-type position effect is the susceptibility of a variegating gene to modification by changes in the euchromatic-heterochromatic balance. GOWEN and GAY (1933) first showed that the variegation of the white gene could be more or less suppressed by adding a Y

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chromosome. Since then, the ability of an extra Y to suppress variegation has been demonstrated for many $R(+)$ euchromatic loci. Several investigators (PANSHIN 1936; KHVOSTOVA 1939) have attempted to determine the effect of an extra Y on the $R(ci^+)$ position effect but a clear-cut answer has never been obtained. SCHULTZ (1936) discovered that variegation resulting from rearrangements of the normal allele of the light gene, lt^+, which lies within or close to the chromocentral heterochromatin of the second chromosome, is also modified by the addition of an extra Y chromosome. In this case the effect is to enhance the variegation of light. Based on these observations a kind of rule has been formulated which states that variegation of euchromatic loci is suppressed by an extra Y chromosome whereas variegation of genes located within or close to heterochromatin is enhanced by an extra Y (MORGAN and SCHULTZ 1942).

In the experiments described below, the influence of an extra Y chromosome on the Dubinin effect has been measured in order to determine whether the Dubinin effect is modified by an extra Y and thus conforms to the V-type position effects. Furthermore, since the *ci* locus lies within or close to the chromocentral heterochromatin, it is of particular interest to determine whether $R(ci^+)$ constitutes the second case of a position effect which is enhanced by the addition of an extra Y chromosome.

The results obtained in these experiments increase the likelihood that the Dubinin effect is a V-type position effect since for each rearrangement tested a highly significant difference exists between females with and without a Y chromosome. Moreover, the modification of the position effect is, in each case, in the direction of suppression.

GENERAL METHODS AND MATERIALS

The expression of $R(ci^+)/ci$ is subject to modification from a wide variety of sources both environmental and genetic. In order to measure the effect of a single variable, in this case the Y chromosome, it is necessary to have control over any modifier genes. To meet this need methods were devised whereby groups of flies with different numbers of Y chromosomes were obtained from a single mating. These methods are described under Experimental Procedure.

The methods which were used also provided for a measurement of the degree of vein interruption a generation before it was possible to know whether the fly carried a Y chromosome. The determination of the presence or absence of a Y in the following generation was nearly always made without knowledge of the *ci* index ascribed to the parent a generation before. In this way subjective errors, if not eliminated entirely, were considerably reduced.

A complication which arises in working with translocation heterozygotes of the fourth chromosome is the tendency of such stocks to become partially triplo-4 unless they are balanced with a fourth chromosome dominant mutant which is lethal when present in two doses. The translocation will cause some nondisjunction between it and the free four producing diplo-4 and nullo-4 gametes and consequently triplo-4 and haplo-4 individuals. Such triplo-4 individuals will,

through preferential segregation of the two normal fours, be maintained to a greater or lesser degree in the stock. If the normal fourth chromosome of such a translocation stock carries the normal allele of *ci*, then on outcrossing to *ci*, both *R(ci⁺)/ci* and *R(ci⁺)/ci⁺/ci* will be tested. Those translocation bearing progeny which carry one ci^+ will not show the Dubinin effect.

Also rearrangements balanced with *ci* ey^R and selected on the basis of non-ey^R will tend to become triplo-4 since this condition will cause a shift of venation toward normality (STERN, MACKNIGHT, and KODANI 1946). On outcrossing such a translocation, the great majority of translocation bearing gametes will carry an extra *ci* ey^R fourth chromosome. The effect of an extra Y or X0 condition on *R(ci⁺)/ci/ci* instead of on *R(ci⁺)/ci* will then be measured.

In the present study, the possibility of this complication was avoided by clearing the stocks of extra fourth chromosomes and then rebalancing in a way that eliminates the possibility of the recurrence of this condition.

MATERIALS AND STOCKS

1. T(3:4)86D: Translocation (3:4)86D. This translocation which was found by E. B. LEWIS, was induced by irradiation of $bx^{34e}e^4$ males with 400 rep units of fast neutrons. The salivary gland chromosome analysis by LEWIS shows a break in 3 just after 86D1–2 and a break in 4 at 101F. The translocation is homozygous viable and the homozygote has normal venation. The translocation was established in a homozygous stock free of extra fours.

2. T(3:4)89E: Translocation (3:4)89E. This translocation which was found by E. B. LEWIS, was obtained from an X-rayed *ss bx* Su^2 *-ss* male. It is inseparable from bxd^{101}, an extreme bithoraxoid mutant. The salivary chromosome analysis by LEWIS shows a break in 3 between 89E1–2 and 89E3–4 doublets and at 101 in chromosome 4. The translocation was balanced against ey^D and freed of extra fourth chromosomes.

3. *In(1)*dl-49,v^{0f}/*In(1)*dl-49,v^{0f}/Y^{C-S}; *ci* ey^R/*ci* ey^R ♀ × +/Y^{C-S}; *ci* ey^R/*ci* ey^R ♂. The *In(1)*dl-49,v^{0f} chromosome was derived from a single crossover between an *In(1)*dl-49,v^{0f}*f* chromosome and a Canton-S chromosome. Thus, the proximal region of the X chromosome, including the centromere, is of Canton-S origin. The symbol Y^{C-S} indicates a chromosome from a Canton-S wild type stock.

4. *ci gvl bt*. This stock was obtained as a crossover by A. H. STURTEVANT and maintained as a homozygous stock since 1951.

5. *ci* sv^n. This stock was obtained as a crossover by A. H. STURTEVANT and maintained as a homozygous stock since 1951.

Mutant or rearrangement symbols used in the tests or discussed below are as follows (for fuller description see BRIDGES and BREHME and Drosophila Information Service Nos. 27 and 28):

al^2 (aristaless²), *B* (Bar), *bt* (bent), *bw* (brown), *bx* (bithorax), bx^{34e} (bithorax ^{34e}), bxd^{101} (bithoraxoid^{101}), *ci* (cubitus-interruptus), *dm* (diminutive), e^4 (ebony⁴), ey^D (eyeless-dominant), ey^R (eyeless-Russian), *FM3* (First

Multiple 3), *gvl* (grooveless), *In(1)*dl-49 (Inversion (1) delta-49), *In(2L)Cy* (Inversion (2L) with Curly), *In(2R)bw*^{VDe1} (Inversion (2R) brown-Variegated of Demerec no. 1), *lt* (light), *M(2)S10* (Minute (2) Schultz' no. 10), *SM1* (Second Multiple 1), *Sb* (Stubble), *sc*^{s} (scute^{s}), *ss* (spineless), *Su*^{s}*-ss* (Suppressor^{s} of spineless), *sv*^{n} (shaven-naked), *sp*^{s} (speck^{s}), *v* (vermilion), *v*^{Of} (vermilion of Offermann), *y*^{s} (yellow^{s}), *y*^{31d} (yellow^{31d}), Y^{C-S} (Y chromosome derived from Canton-S stock), *Xa* (Xasta).

Classification

The classification of the degree of cubitus interruption is that used by STERN, SCHAEFFER and HEIDENTHAL (1946). The portion of the fourth vein examined was that distal to the posterior cross vein. A completely normal vein was given a value of 0; a thinning at one or several points but no discernible break was given a value of 1; the absence of the vein for any distance up to a maximum of one half was classified as 2; a break greater than one half and no greater than seven eights the vein length was classified as 3. Retention of one eighth or less was valued at 4.

Each wing was graded as a unit and the two values for each female tested were added. Nine classes were set up extending from 0–8.

EXPERIMENTAL PROCEDURE AND RESULTS

Effect of one extra Y chromosome on R(ci^{+})/ci: Single virgin females whose genotype was *In(1)*dl-49,*v*^{Of}*/In(1)*dl-49,*v*^{Of}Y^{C-S}; *ci ey*^{R}*/ci ey*^{R} were mated in vials to single males which were homozygous for T(3:4)86D or to single males which were heterozygous for T(3:4)89E. The vials were kept at 25°C for 24 hours and then the flies were transferred to bottles which were kept at 20° C until hatching. The regular translocation bearing female progeny of such matings consist of two types which are phenotypically indistinguishable. These are:

Type 1. *In(1)*dl-49,*v*^{Of}/+; T(3:4)/+; *ci ey*^{R}

Type 2. *In(1)*dl-49,*v*^{Of}/+/Y^{C-S}; T(3:4)/+; *ci ey*^{R}

Thus two groups of daughters are obtained from a single mating, one of which carries an extra Y chromosome. In all other respects the two groups are genotypically alike since heterozygosity in the genomes of the parents may be expected to be randomized among the offspring. Also since the two groups are grown in the same bottle, their environment may be considered identical. If a significant difference in the expression of *R(ci*$^{+}$*)/ci* is observed between the two groups, the difference can be attributed to the Y chromosome.

All Type 1 and Type 2 females were collected within ten-hour intervals to assure virginity, numbered for identification and then classified as to the degree of cubitus vein interruption in the manner described above. Each female was then progeny tested by mating her singly to several *B;Sb/Xa* males in order to determine whether she carried an extra Y chromosome. The presence of the dominant marker, Bar, in the X chromosome of the father served to distinguish between those progeny which were regular for the disjunction of the X chromo-

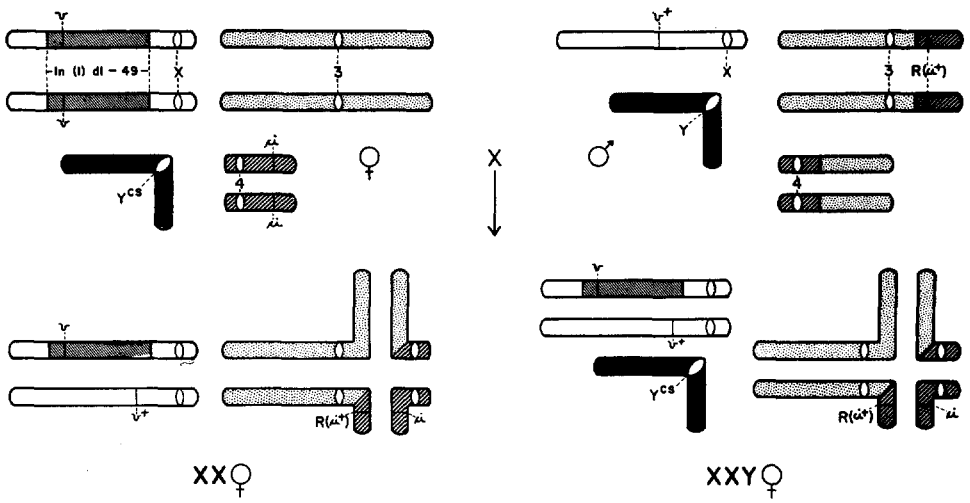

FIGURE 1.—Mating scheme for the production of two groups of daughters which are genotypically equivalent except for the presence or absence of an extra Y chromosome. P_1, *In(1)*dl-49,v^{of}/*In(1)*dl-49,v^{of}/Y^{c-s}; *ci* ey^R/*ci* ey^R ♀ × *R(ci⁺)*/*R(ci⁺)* ♂; F_1 females, Type I. *In(1)* dl-49,v^{of}/+; *R(ci⁺)*/+; *ci* ey^R Type II. *In(1)*dl-49,v^{of}/+/Y^{c-s}; *R(ci⁺)*/+; *ci* ey^R.

somes and those progeny which resulted from nondisjunction of the X's. Regular progeny consisted of *B* females and B^+ males whereas nondisjunctional progeny consisted of B^+ females and *B* males. The presence of the dominant marker *Sb* in one of the third chromosomes and of *Xa* in the other third chromosome of the male parent served as a check on the virginity of the female parent. All of the progeny were expected to carry *Sb* or *Xa* and any failure to do so would indicate that the mother was nonvirgin.

It is well known that the frequency of nondisjunctional progeny of females heterozygous for the dl-49 inversion is markedly altered by the addition of a Y chromosome. STURTEVANT and BEADLE (1936) have shown that an XXY female heterozygous for the dl-49 inversion in X produces 45.6 percent secondary nondisjunctional progeny. In contrast, a female heterozygous for dl-49 without a Y produces only 0.1 percent primary nondisjunctional offspring. The difference in the percent of nondisjunctional progeny from females heterozygous for the dl-49 inversion served as the basis for recognizing the presence or absence of a Y chromosome. If the exceptional progeny approached 50 percent, the mother was scored as having a Y. In general no more than two percent of exceptional progeny were observed from mothers scored as XX. The highest number of exceptions observed from such a mother was approximately five percent (8/158). STURTEVANT (MORGAN and STURTEVANT 1944) observed that the presence of a heterozygous inversion in an autosome coupled with a heterozygous inversion in the X will increase the amount of primary nondisjunction and decrease the

amount of secondary nondisjunction of the X chromosomes. A heterozygous translocation coupled with a heterozygous inversion in X appears to have a similar effect. This would explain the deviation from the expected in those cases in which the exceptional progeny arising from primary nondisjunction were too high. This effect was never sufficient to cause any ambiguity in the results and no culture was discarded because of uncertainty as to whether the mother did or did not possess a Y chromosome.

In the case of T(3:4)86D six cultures or one percent were discarded for sterility. All fertile cultures produced haplo-4 flies indicating that both parents were diplo-4. Nonvirginity was absent. The results of this experiment, which are given in Table 1, show that a highly significant difference exists between females with and without a Y chromosome. Furthermore, the effect of the extra Y is partially to suppress the Dubinin effect.

In the case of T(3:4)89E eleven cultures or three percent were discarded for sterility. All fertile cultures produced haplo-4 flies. Nonvirginity was absent. The results of this experiment are given in Table 2. The difference between XX and XXY females is highly significant and, again, the effect of the extra Y is partially to suppress the Dubinin effect.

Comparison of the effect of one and two extra Y chromosomes on R(ci+)/ci: In a second experiment, a comparison of the effect of one and two extra Y chromosomes on the expression T(3:4)86D, *bx*^{34e}*e*^{4}*/ci* was studied. In order to obtain females which carry two extra Y chromosomes, it is necessary to secure

TABLE 1

Distribution according to degree of cubitus interruptus of females of constitution In(1)*dl-49*,v^{0f}/+; *T(3:4)86D*,bx^{34e} e^{4}/+; ci ey^{R} *with and without an extra Y chromosome*

Sex chromosomes	Degree of cubitus interruption* 0	1	2	3	4	5	6	7	8	Totals
XX	14	26	64	56	53	26	16	0	0	255
XXY	30	63	82	41	41	21	12	1	0	291

$\chi^2 = 25.89$. n = 6†. P < .0003.

* For classification, see text. 0 indicates normal for both wings. 8 indicates one eighth or less of the vein for both wings.
† Classes 6 and 7 were combined in calculating χ^2.

TABLE 2

Distribution according to degree of cubitus interruptus of females of constitution In(1)*dl-49*,v^{0f}/+; *T(3:4)89E*,ss bx bxd/+; ci ey^{R} *with and without an extra Y chromosome*

Sex chromosomes	Degree of cubitus interruption* 0	1	2	3	4	5	6	7	8	Totals
XX	3	3	9	20	44	55	41	5	0	180
XXY	9	13	25	37	37	31	16	1	0	169

$\chi^2 = 42.20$. n = 6†. P < .0001.

* See Table 1.
† Classes 6 and 7 were combined in calculating χ^2.

parents, each of which are known to carry one extra Y. This was accomplished by introducing into one of the second chromosomes of each parent the inversions *(2L)Cy*, *Cy* and *(2R)bw^{VDe1},bw^{VDe1}*. The suppression of the dominant brown-variegated phenotype by an extra Y chromosome so as to produce a *Cy* fly with suppressed brown variegation served as the means for identifying the presence of an extra Y chromosome in each parent. Many males and females of the following genotypes

$$\frac{y^2v}{FM3,y^{s1d}sc^8dm\ B\ l}\ Y^{c-s};\ \frac{Ins(2L)Cy,\ (2R)bw^{VDe1},Cy\ bw^{VDe1}}{bw};\ \frac{ci\ sv^n}{ci\ sv^n}\ ♀$$

$$\times\ y^2v/Y^{c-s}/Y^{c-s};\ \frac{Ins(2L)Cy,\ (2R)bw^{VDe1},\ Cy\ bw^{VDe1}}{bw};\ \frac{T(3:4)86D,\ bx^{34e}e^4}{+\ ;\ ey^D}\ ♂$$

were pair mated in vials, kept at 25°C for 24 hours and then transferred to bottles. The bottles were kept at 20°C until the offspring hatched.

The non-Bar, non-Curly, translocation-bearing female progeny of such a mating consists of three types which are phenotypically indistinguishable.

These are:

Type I. y^2v/y^2v; *bw/bw* T(3:4)86D/*ci sv^n*

Type II. $y^2v/y^2v/Y^{c-s}$; *bw/bw;* T(3:4)86D/*ci sv^n*

Type III. $y^2v/y^2v/Y^{c-s}/Y^{c-s}$; *bw/bw;* T(3:4)86D/*ci sv^n*

The three types arise in each case from the mating of a single male and female. Consequently, the environmental and genotypic background of each group is equivalent.

Females of Type I, Type II, and Type III were collected within ten-hour intervals to assure virginity, were numbered, graded for degree of cubitus vein interruption and individually mated to *v;bw^{VDe1}/SM1, al^2 Cy sp^2* males. The presence of the yellow^2 (y^2) mutant in both X chromosomes of the female parent and of y^+ in the X chromosome of the male parent served to distinguish between those progeny which arose from the regular disjunction of the X chromosomes and those progeny which arose from the nondisjunction of the X chromosomes. The regular progeny consist of y^+ females and y^2 males, whereas the nondisjunctional progeny consists of y^2 females and y^+ males.

The presence of vermilion (*v*) in both parents and of brown (*bw*) in the female parent served to facilitate the detection of the suppression of variegation. Females which are *v/v;bw^{VDe1}/bw* or males which are *v*/Y;*bw^{VDe1}/bw* have nearly white eyes whereas the addition of an extra Y to either of these genotypes gives a red eye color which is indistinguishable from that of vermilion.

The method of determining the number of Y chromosomes in Type I, Type II, and Type III females is to observe the proportion of *bw^{VDe1}* (non-Curly) progeny of such a female which shows suppression of variegation.

If the female is Type I (XX) then all the *bw^{VDe1}* regular progeny are expected to have unsuppressed brown variegation.

If the female is Type II (XXY) then the *bw^{VDe1}* regular progeny are expected to consist of approximately equal numbers of suppressed and unsuppressed flies.

If the female is Type III (XXYY) then most of the bw^{VDe1} regular progeny are expected to have suppressed brown variegation (STERN 1929).

Eighty-seven females were progeny tested and of these seven or eight percent were sterile. Among the remaining 80 flies, seven flies or nine percent were classified as Type I (XX), 46 or 58 percent were classified as Type II (XXY) and 27 or 34 percent were classified as Type III (XXYY).

Since the females were in all other respects genotypically alike it was possible to compare the effect of no Y, one Y, and two Y chromosomes on their fertility. The average number of non-Curly progeny from an XX mother was 230; from an XXY mother was 146; and from an XXYY mother was 34. Thus fertility in the female appears to vary inversely with hyperploidy for the Y chromosome.

All of the Type I and Type II females produced bw^{VDe1} haplo-4 flies. Four of the Type III females produced no bw^{VDe1} haplo-4 flies. In view of the small total numbers of bw^{VDe1} progeny from XXYY females, the absence of the class is occasionally expected. The number of progeny nondisjunctional for the X's was less than two percent for each type.

The results of this experiment, which are given in Table 3, show that a highly significant difference exists between females with one Y chromosome (Type II) and those with two Y chromosomes (Type III). The effect of two extra Y's is to suppress the Dubinin effect to a greater extent than does one extra Y chromosome. The number of Type I (no extra Y) females was too small to permit a statistical analysis of this group.

Effect of one extra Y chromosome on homozygous ci: Although the addition of one or two extra Y chromosomes partially suppresses the Dubinin effect, it is possible that extra Y's might have a similar effect on the *ci* mutant. At least one mutant, sparkling (L. V. MORGAN) which is unlocated in chromosome 4, is known to behave in this way. XX females and XO males exhibit the sparkling characteristic whereas XXY females and XY males do not. It seems unlikely that a Y chromosome would have a similar effect in the case of *ci* since STERN, MACKNIGHT and KODANI (1946) reported that homozygous *ci* males have a more extreme **ci** phenotype than homozygous *ci* females. Nevertheless, the possibility was tested in the following experiment.

Females of the genotype *In(1)*dl-49,v^{of}/*In(1)*dl-49,v^{of}/Y^{c-s};*ci* ey^R/*ci* ey^R were

TABLE 3

Distribution according to degree of cubitus interruptus of females of constitution y^2v/y^2v; bw/bw; *T(3:4)86D*,bx^{34e} e^4/+; ci sv^n *with one or two extra Y chromosomes*

Sex chromosomes	Degree of cubitus interruption*									Totals
	0	1	2	3	4	5	6	7	8	
XXY	1	1	2	1	5	7	28	1	0	46
XXYY	1	2	1	4	7	8	4	0	0	27
$\chi^2 = 16.19$.	n = 3†.		P < .001.							

* See Table 1.
† Classes 0–3 and classes 6 and 7 were combined in calculating χ^2.

mated to males homozygous for *ci gvl bt*. F_1 virgin females whose genotype was *In(1)*dl-49,v^{of}/+; *ci* ey^R/*ci gvl bt* or *In(1)*dl-49,v^{of}/+/Y^{c-s}; *ci* ey^R/*ci gvl bt* were numbered, graded and mated singly to *B; Sb/Xa* males to determine if an extra Y chromosome was present. Six cultures or 2.4 percent were discarded for sterility. Nonvirginity was absent.

The results of this test, which are given in Table 4, show that an extra Y chromosome has no significant effect on the expression of the homozygous *ci* mutant.

DISCUSSION

The results which have been obtained in these experiments show that the phenotype of position effects of *R(ci⁺)* is susceptible to modification by extra Y chromosomes. The degree of modification is greater in the presence of two extra Y chromosomes than in the presence of one extra Y. The homozygous *ci* mutant, on the other hand, is not significantly affected by the addition of an extra Y. This evidence, in conjunction with the euchromatic-heterochromatic type of rearrangement which is known to be involved in these translocations, greatly increases the likelihood that the Dubinin effect is a variegated type position effect.

GOWEN and GAY (1933) discovered that the addition of an extra Y chromosome suppresses the variegation of a euchromatic locus. This suppression has since been confirmed for all variegating euchromatic loci which respond to changes in the number of Y chromosomes and has been generalized into a rule (MORGAN and SCHULTZ 1942). Contrariwise, position effects for rearrangements of the light gene, whose locus lies either within or close to proximal heterochromatin of chromosome 2, are enhanced by an extra Y and suppressed in the XO condition (SCHULTZ 1936). Since light is the only heterochromatic locus for which the effect of different doses of the Y chromosome is definitely known and since the Y effect is in this case in the direction opposite to that observed for euchromatic loci, a second "rule" has emerged which states that variegation of "heterochromatic" loci is enhanced when an extra Y is added and suppressed the XO condition (MORGAN and SCHULTZ 1942).

This "rule" would have much greater validity if the behavior of other "heterochromatic" loci was found to resemble that of light. Actually, with the exception

TABLE 4

Distribution according to degree of cubitus interruptus of females of constitution In(1)*dl-49*,v^{of}/+; ci ey^R/ci gvl bt *with and without an extra Y chromosome*

Sex chromosomes	Degree of cubitus interruption* 0	1	2	3	4	5	6	7	8	Totals
XX	0	0	6	15	18	36	30	5	0	110
XXY	0	0	2	9	20	42	38	11	4	126
$\chi^2 = 8.59$.	n = 4†.		P = .075.							

* See Table 1.
† Classes 2 and 3 and classes 7 and 8 were combined in calculating χ^2.

of *lt* and *ci* little is known concerning position effects of "heterochromatic" loci in *D. melanogaster*. This is due, at least in part, to the facts that such loci are extremely limited in number; that the phenotype of some of these is unsuitable for the study of variegation; and that in at least one case, that of straw, efforts to detect variegation have been unsuccessful (GRELL, unpublished data).

SCHULTZ (MORGAN and SCHULTZ 1942) found that M(2)S10, when heterozygous for the mutant rolled which is located in or close to the proximal heterochromatin of 2R, gave a variable phenotype for rolled. SCHULTZ suggested that if the deficiency of M(2)S10 does not include the locus of rolled, the removal of the heterochromatic block might bring rolled closer to euchromatin and thus cause it to show a position effect. If the behavior of rolled resembles that of light, the addition of an extra Y would be expected to enhance this effect. According to SCHULTZ, such an addition did not affect the manifestation of rolled. Since it is possible that the deficiency for M(2)S10 does include the locus of rolled, this case may not be cogent.

BAKER (1953) reported on the effect of an extra Y chromosome on a *R*(+) of the peach gene which is located within or close to the proximal heterochromatin of chromosome 5 in *D. virilis*. He found that the extra Y had no striking effect on the variegation of this rearrangement although a slight suppression might occur. As BAKER points out, this is not strictly comparable to the light case since the extra Y is added to a Y:5 translocation which is a heterochromatic-heterochromatic rearrangement rather than a heterochromatic-euchromatic rearrangement.

Although position alleles of *ci*+ have been studied extensively, the effect of changes in the euchromatic-heterochromatic balance on such position alleles has not been determined with any degree of certainty. Thus KHVOSTOVA (1939) reports that in four cases of *R*(+) of *ci*, the *ci*-index was more extreme when an extra Y was added while in two cases it was less so. She concludes that her data were insufficient and that the problem required further study with more refined methods.

The present experiments were undertaken in the knowledge that the Dubinin effect is difficult to study for a number of reasons. Chief among these are the susceptibility of *ci* position effects to modification by both genetic and environmental factors and the likelihood that fourth chromosome translocation stocks are partially triplo-4. As a preliminary step to these investigations, the stocks which were to be used were cleared of extra fourth chromosomes and were rebalanced so as to prevent the recurrence of this condition. The experiments were planned so that a comparison could be made between populations of otherwise genetically equivalent females which carried different "doses" of the Y chromosome. The populations which were to be compared were reared in the same bottles under carefully controlled temperature conditions. In order to minimize subjective errors, the *ci*-index of the female was determined a generation before her Y constitution was known. Likewise, the Y determination was nearly always made without the knowledge of her *ci* classification.

The results of these experiments show that the Y chromosome partially suppresses the Dubinin effect. A suppression is observed when XXY females are compared with XX females and when XXYY females are compared with XXY females. Although the effect of the XO condition on *R(ci⁺)/ci* has not been studied it is of interest to note that ALTORFER (1952) compared the influence of the XY and XO condition on *R(ci)/ci*. She observed that those *R(ci)* which respond to changes in the Y constitution show an enhancement in the XO condition. This finding is consistent with the result which would be expected on the basis of the present experiments.

Thus extra Y chromosomes modify the Dubinin effect in the same way that they modify the variegation of euchromatic loci and not as they modify position effects of the light gene. Clearly then, on the basis of our present knowledge, no generalization can be made concerning the effect of an extra Y chromosome on the position effect of genes located in or near heterochromatin.

SUMMARY

1. Groups of females which were genotypically equivalent except for the presence or absence of an extra Y chromosome were compared for the expression of *R(ci⁺)/ci*. A highly significant difference was observed between the group with and the group without an extra Y in the cases of the two rearrangements which were studied. The effect of the extra Y in both cases was to partially suppress the Dubinin effect.

2. Two groups of females which were genotypically equivalent except for the presence of one or two Y chromosomes were compared for the expression of *R(ci⁺)/ci*. A highly significant difference was observed between the two groups. The group with two extra Y chromosomes showed a greater suppression of the Dubinin effect.

3. Two groups of females which were genotypically equivalent except for the presence or absence of an extra Y chromosome were compared for the expression of homozygous *ci*. No significant difference was observed between the two groups.

4. The responce of the phenotypic expression of *R(ci⁺)/ci* to changes in the euchromatic-heterochromatic balance greatly strengthens the case for identifying the "Dubinin effect" as a V-type position effect.

5. The Dubinin effect, a position effect for the "heterochromatic" locus *ci*, resembles V-type position effects of euchromatic loci in that it is partially suppressed by extra Y chromosomes. This observation is not consistent with the generalization, based on the case of light, which states that position effects of "heterochromatic" loci are enhanced by the addition of extra Y chromosomes.

6. A comparison of otherwise genotypically equivalent females which carried no extra Y, one extra Y and two extra Y chromosomes showed a highly significant difference in the fertility of the three groups. The fertility of the females varies inversely with hyperploidy for extra Y chromosomes.

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LITERATURE CITED

ALTORFER, N., 1952 Influence du chromosome Y sur l'expression de la mutation cubitus interruptus chez *Drosophila melanogaster*. Ann. Soc. Zool. Belg. Louvain, **82** (1951): 447–462.

BAKER, W. K., 1953 V-type position effects of a gene in *Drosophila virilis* normally located in heterochromatin. Genetics **38**: 328–344.

DUBININ, N. P., and B. N. SIDOROV, 1934 Relation between the effect of a gene and its position in the system. Am. Naturalist **18**: 377–381.

GLASS, H. B., 1933 A study of dominant mosaic eye-color mutants in *Drosophila melanogaster*. J. Genet. **28**: 69–112.

GOWEN, J. W., and E. A. GAY, 1933 Eversporting as a function of the Y-chromosome in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. **19**: 122–126.

GRELL, R. F., 1956 Influence of a Y chromosome on the Dubinin effect. Genetics **41**: 645–646.

HANNAH, A., 1951 Localization and function of heterochromatin in *Drosophila melanogaster*. Advances in Genet. **4**: 87–125.

KHVOSTOVA, V. V., 1939 The role played by the inert chromosome regions in the position effect of the cubitus interruptus gene in *Drosophila melanogaster*. Bull. Acad. Sci. U.R.S.S.: 541–574.

LEWIS, E. B., 1950 The phenomenon of position effect. Advances in Genet. **3**: 73–115.

MORGAN, L. V., 1947 A variable phenotype associated with the fourth chromosome of *Drosophila melanogaster* and affected by heterochromatin. Genetics **32**: 200–219.

MORGAN, T. H. and J. SCHULTZ, 1942 Investigations on the constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Ybk. **41**: 242–245.

MORGAN, T. H. and A. H. STURTEVANT, 1944 Maintenance of a Drosophila stock center, in connection with investigations on the constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Ybk. **43**: 164–165.

MULLER, H. J., 1930 Types of visible variations induced by X-rays in Drosophila. J. Genet. **22**: 299–334.

PANSHIN, I. B., 1936 A demonstration of the specific nature of position effect. Compt. Rend. (Dokl.) Acad. Sci. U.R.S.S. **1**: 83–86.

SCHULTZ, J., 1936 Variegation in Drosophila and the inert chromosome regions. Proc. Natl. Acad. Sci. U.S. **22**: 27–33.

STERN, C., 1929 Untersuchungen über Aberrationen des Y-Chromosoms von *Drosophila melanogaster*. Z. ind. Abst. Vererb. **51**: 253–353.

STERN, C., R. H. MACKNIGHT, and M. KODANI, 1946 The phenotypes of homozygotes and hemizygotes of position alleles and of heterozygotes between alleles in normal and translocated positions. Genetics **31**: 598–619.

STERN, C., E. W. SCHAEFFER, and G. HEIDENTHAL, 1946 A comparison between the position effects of normal and mutant alleles. Proc. Natl. Acad. Sci. U.S. **32**: 26–33.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.