EFFECT OF THE ABSENCE OF THE Y CHROMOSOME ON
THE EXPRESSION OF THE CUBITUS INTERRUPTUS
PHENOTYPE IN VARIOUS TRANSLOCATIONS
IN *DROSOPHILA MELANOGASTER*

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IN 1934, DUBININ and SIDOROV showed that in many translocations of the fourth chromosome, the dominance of the normal allele of the cubitus interruptus (*ci*) gene was reduced; $R(ci^+)/ci$ flies were mutant. Work by DUBININ, SOKOLOV and TINIAKOV (1935), KHWOSTOVA (1936, 1939) and STERN and collaborators (1943, 1955; see the list of references in STERN and KODANI 1955) showed that this position effect, the *Dubinín effect* (STERN and HEIDENTHAL 1944) depends usually upon a translocation of the gene from a region within or close to the heterochromatin of the fourth chromosome to a euchromatic region from another chromosome. This same modification of the euchromatic-heterochromatic relations in the immediate vicinity of the gene is the condition which also determines the variegated type position effect, as was shown by GOWEN and GAY (1934), MULLER (1935), SCHULTZ (1936), and MORGAN, BRIDGES and SCHULTZ (1938).

SIDOROV (1941) and STERN and HEIDENTHAL (1944) showed that cytological rearrangements of the mutant *ci* allele produce exaggerated mutant phenotype when opposite a fourth chromosome containing the mutant *ci* gene. Yet, the same translocations in the homozygous or hemizygous condition result in a normal or nearly normal phenotype, a particular feature also encountered for the Dubinín effect. For this reason, the term Dubinín effect, which was originally applied only to comparisons of $R(ci^+)/ci$ with ci^+/ci will be used in a broader sense in the present paper to include comparisons of $R(ci)/ci$ with ci/ci . However, the analysis conducted by STERN and KODANI (1955) of an unselected sample of $R(ci)$ translocations has shown that position effects of low expression may be produced by several types of chromosomal reassortment in the vicinity of the *ci* gene, other than a clearcut euchromatin-heterochromatin fusion.

In his study of position effect, LEWIS (1950) has considered, with justifiable caution, the Dubinín effect as a variegated-type position effect of a gene located in heterochromatin. Gene expressivity being highly variable for the *ci* mutant, it is impossible, however, to distinguish between this phenotypic variability and a mosaic effect due to change of position of the gene. But, if we are really dealing with a variegated-type position effect, the Dubinín effect should be sensitive to the typical modifying factors of this phenomenon; essentially to the presence of

the Y chromosome. For instance, variegation of the light (*lt*) phenotype, a heterochromatic gene position effect, is exaggerated in the presence of a supernumerary Y chromosome and suppressed in X0 males (SCHULTZ and DOBZHANSKY 1934).

GRELL (1959) has shown in the case of two $R(ci^+)$, 3;4 translocations, that the Dubinin effect conforms to the variegated-type position effect in being influenced by the dose of Y chromosome in the nucleus. She tested these translocations (T86D and T89E) for their phenotypes in XX and XXY constitutions and observed a significant reduction of the position effect in the presence of one supernumerary Y chromosome. Addition of a second Y chromosome gives greater suppression. In the same conditions, the *ci/ci* female controls show no significant modification. There are no data concerning the effect of the absence of the Y chromosome on $R(ci^+)/ci$.

$R(ci)$ translocations with Dubinin effect have not been tested in the presence of one or two additional Y chromosomes.

In the present work, the effect of the absence of the Y chromosome has been considered not only in the case of the $R(ci)$ Dubinin effect, but also in the case of $R(ci)$ translocations of other types, in the same $R(ci)/ci$ combination. Most of these data were contained in a paper published in 1951; unfortunately this publication (ALTORFER 1951) is not readily accessible to many readers and it is necessary to give the data here in concise form in order to make the present analysis intelligible.

MATERIALS AND METHODS

The *ci* and $R(ci)$ stocks were obtained from the laboratory of DR. C. STERN. For the detailed description of the *ci* gene and its position alleles we refer the reader to the papers of STERN and collaborators.

The *ci* gene (BRIDGES and BREHME 1944) is characterised by one or many interruptions of the fourth or cubital vein, essentially in the region distal to the posterior crossvein. Expression and penetrance are highly variable under influence of external factors, especially of temperature. By genetical and cytological data, it is possible to locate the *ci* gene in the fourth chromosome, at the limit of the heterochromatin, in band 102B1 or in its immediate vicinity (STERN and KODANI 1955).

The quantitative estimation of the phenotype has been obtained by appraising the degree of interruption of the cubital vein in its distal region (Figure 1). Six classes have been distinguished, as specified in the legend to Figure 1. The degree of expression in each individual is based on the average interruption of both wings. The mean of the distribution is calculated, giving each class a weight corresponding to its designation. Expression of the character differs with sex. Accordingly, the sexes are always classified separately.

Of course, this linear scale is only an approximation and other possible scales might lead to different conclusions.

Translocations: The translocations utilised derive from the series of 55 $R(ci)$ translocations obtained and studied by STERN (1943-1955). They are simple reciprocal translocations between chromosome 2 or 3 and chromosome 4 carrying the *ci* and ey^+ (eyeless) gene, the break in the latter case being in the vicinity of the *ci* locus. Positions of the breaks in chromosomes 2, 3 and 4 have been analysed by KODANI (STERN and KODANI 1955), and are given for the different rearrangements in Table 1. Those rearrangements are designated, following the notation introduced by STERN, by the $Rx(ci)$ symbol, where *x* stands for a number giving the order of discovery of the rearrangements. All the stocks had been made relatively isogenic with each other and the wild Canton-S stock, except for the small fourth chromosome. As the following experiments

FIGURE 1.—Wing phenotypes varying in the degree of interruption of the cubital vein. (Adapted from STERN 1943.) Six classes have been distinguished:

Class 0: Fourth vein normal (a).

Class 1: Thinning of the fourth vein in one or many sites, or interruption of this vein no longer than the width of the vein (b).

Class 2: One or many interruptions of the fourth vein, the total length of the interruptions being less than half the total length of the fourth vein (c-d).

Class 3: One or many interruptions of the fourth vein, the total being more than half the total length of the fourth vein but leaving a small portion distal to the crossvein, longer than the width of the vein (e-f).

Class 4: Presence of a small distal portion of vein not longer than the width of the vein, or total absence of the distal fourth vein (g-h).

Class 5: Total absence of the distal fourth vein, with shortening of the anterior crossvein and suppressing the angle formed by the anterior fourth vein and the posterior crossvein (i).

TABLE 1

Relation between position of breaks and degree of position effect in R(ci)/ci translocations (after STERN and KODANI 1955)

Translocation	Break in chromosome 2 or 3*	Break in chromosome 4†	Position effect			
<i>R5(ci)</i>	3L after 69E2	Immed. before 102B1	generally marked			
<i>R8(ci)</i>	3L after 69F2	101				
<i>R31(ci)</i>	3L immed. before 75A1	101				
<i>R23(ci)</i>	3R immed. after 92E2	101F				
<i>R32(ci)</i>	3R immed. after 92E2	Immed. before 102A1				
<i>R29(ci)</i>	3R immed. after 94B2	101				
<i>R4(ci)</i>	3 in centromeric region	101‡	low or absent			
<i>R28(ci)</i>	3 in centromeric region	101				
<i>R35(ci)</i>	3L 79F	101				
<i>R25(ci)</i>	3R 81F	101				
<i>R17(ci)</i>	3L immed. before 67E1	Immed. before 102C1	<table> <tr> <td rowspan="3">} posi- tive</td> <td rowspan="5">} generally low</td> </tr> <tr></tr> <tr></tr> <tr> <td rowspan="2">} nega- tive</td> </tr> <tr></tr> </table>	} posi- tive	} generally low	} nega- tive
} posi- tive	} generally low					
} nega- tive						
<i>R40(ci)</i>	3R before 94A1	before 102F2				
<i>R22(ci)</i>	3R after 98C1	after 102B1				
<i>R41(ci)</i>	3R between 98C and D	before 102C1				
<i>R36(ci)</i>	2R immed. after 47A1	immed. before 102C1				
<i>R45(ci)</i>	2R 58D	after 102D-E				

* Chromosome-2 centromere is at 40; 3-centromere is at 80.

† *ci* is at 102B1; 4-centromere is at 101.

‡ The break is right of the centromere and left of region 101F.

were mainly conducted between 1948 and 1952, no control of the structure of the translocation has been done, unless it was called for by some peculiarity of expression of the *ci* character, as in the case of *R31(ci)*. Later on a few stocks have been retested genetically and cytologically (*R8(ci)*, *R23(ci)*, *R52(ci)*). The stock *R52(ci)* was found to have changed its composition (see RESULTS).

Two types of translocations may be distinguished according to the breakage point in chromosome 4. These two types are also in part distinguishable by the phenotype they yield in heterozygotes of the type *Rx(ci)/ci*. If these heterozygotes give the same mean expression as *ci/ci* homozygotes, no position effect is observed. If they deviate more from normal than *ci/ci*, a positive position effect is said to be observed, and if they deviate less than *ci/ci*, a negative position effect.

In the first type, the break is to the left of the *ci* locus; the distal region of chromosome 4, with *ci*, has fused with a broken end of chromosome 2 or 3. This latter break may be in a more or less distal region, clearly euchromatic, producing a strong positive position effect, the so-called Dubinin effect; or it may be in the centromere region, or immediately adjacent to the heterochromatic region, yielding respectively a weak positive position effect or none at all.

In the second type, the break has occurred to the right of the *ci* locus; a distal part of 2 or 3 has been translocated to the proximal part of 4. The result is a weak and positive effect for translocations 3;4, but the six 2;4 translocations of this type studied by STERN and KODANI (1955), all show a negative position effect.

The stocks were kept as heterozygotes for the fourth chromosome with the *ci ey* genes. Flies carrying the translocation are recognised by the presence of normal eyes produced by the dominant *ey*⁺ gene closely linked to the *ci* gene in the translocation.

ci ey^R stock: This stock has been isolated from the heterozygous translocation *R52(ci)/ci ey*^R and is isogenic with the wild-type Canton-S. The presence of the *ey*^R gene has no influence on the expression of the *ci* phenotype, as has been demonstrated by STERN and KODANI (1955).

Translocation R1(ci)Y: The chromosome 4 carrying the *ci ey*⁺ alleles has been translocated

to the Y chromosome. Males possess generally, beside this translocated fourth chromosome, two normal fourth chromosomes, carrying the $ci\ ey^R$ alleles. Because nondisjunction of the X and Y occurs in the males with a relatively high frequency, this stock gives rise to gametes lacking the sex chromosome.

$\bar{y}$; $ci\ ey^R$ stock: This attached-X stock was obtained from an attached-X yellow (y) strain and made isogenic by repeated mating with males of the Canton-S stock. This line was subsequently made homozygous for $si\ ey^R$, by crossing with the isogenic $ci\ ey^R$ strain. The y attached-X chromosomes will be designated $\bar{y}$ below.

Crosses made to obtain X0 and XY males: A. General method. A comparison will be made between normal and X0 males, of the degree of interruption of the fourth vein elicited by specific translocations in the heterozygous state. The action of the mutant gene is very sensitive to external conditions. It is therefore necessary to compare the phenotype of the translocation to that of a control, for example the homozygote $ci\ ey^R/ci\ ey^R$, obtained in the same culture. The difference between the expressivity of ci in these two genotypes, $R(ci)/ci\ ey^R$ and $ci\ ey^R/ci\ ey^R$, will express the position effect due to the translocation, and may be called *expressivity index* (cf. STERN and KODANI 1955). As the phenotype of the ci homozygote is unchanged in the absence of the Y chromosome (GRELL 1959; and see further), the difference between the expressivity indexes in the X0 and XY conditions will give an estimation of the influence of the genotype on the position effect.

The $ci\ ey^R/ci\ ey^R$ controls are obtained in crosses of $Rx(ci)/ci\ ey^R$ by $ci\ ey^R/ci\ ey^R$, so that the progeny consists of equal numbers of translocation heterozygotes and of $ci\ ey^R$ homozygotes. X0 males are obtained among the progeny of exceptional females from the attached-X stock, without Y chromosome. Those exceptional females appear in the progeny of $\bar{y}$; $ci\ ey^R$ females crossed to $R1(ci\ ey^+)$ Y males with a frequency of 1.7% in our experiments. These exceptional females are eyeless, in contrast to the normal females which carry the ey^+ gene linked to the Y chromosome and so have round eyes. Each exceptional female is mated to a heterozygous $R(ci)/ci\ ey^R$ male from the translocation to be tested. From this mating, we obtain sterile X0 male progeny consisting of $ci\ ey^R/ci\ ey^R$ homozygotes and $R(ci)/ci\ ey^R$ heterozygotes. To obtain XY males for the purpose of comparison, the same $R(ci)/ci\ ey^R$ males used in the previously described cross are individually mated to $\bar{y}$; $ci\ ey^R$ females.

For each translocation, an average of ten pairs of experimental and control cultures was studied. Pairs of bottles where the number of individuals for one of the genotypes in one bottle was less than five, or which contained fertile males, were discarded. All cultures were raised in the same incubator at controlled temperature of $25^\circ \pm 0.5^\circ\text{C}$.

Replications of specific matings sometimes showed a rather high variability. Part of this variability is environmental. Our experiments are based on the assumption that controls and $R(ci)/ci$ heterozygotes respond in the same way to variations in the external conditions, although this fact has not been proved. Thus, we assume that the expressivity index which measures the position effect is little or not at all affected by the environment. There is also genotypic variability. Although strains have been made isogenic at the beginning of the experimental work, a certain uncontrolled genic heterogeneity remains. To reduce this part of the variability to a minimum, we took into account only pairs of cultures derived from the same male. Each replication of the same series of mating is listed as first, second series.

Data provided by the different pairs of cultures have been summarised, and the mean and variance of the total distribution calculated. Let us call d_0 the expressivity index for the X0 males and d_Y the expressivity index for XY males. The difference between these d_0 and d_Y values will give an estimation of the influence of the Y chromosome on the position effect. We considered as significant, deviations for which the t value, $t = (d_0 - d_Y) / \sigma(d_0 - d_Y)$ is higher than 2. In this expression, the denominator is the square root of the sum of the standard errors calculated on the interruption means of the four compared genotypes, divided respectively by the number of items in each genotypic class.

B. Modified method. In some cases, we modified the genetic method for the purpose of eliminating errors due to culture conditions.

Attached-X females with no Y chromosome and with one Y chromosome, were crossed succes-

TABLE 2

Influence of the absence of the Y chromosome on position effect of the 3;4, R(ci)/ci translocations

<i>R(ci)</i> No.	Break in chromosome		Position effect	Expressivity index		$(d_0 - d_Y)$	t-value
	3	4		X0 $=d_0$	XY $=d_Y$		
5*	In	To the	Generally	+0.64*	+1.01*	-0.37*	2.97*
8	euchromatic	left	strong	+1.56	+1.26	+0.30	1.45
31*	region	of <i>ci</i>		-1.23*	-0.95*	+0.28*	3.50*
23				+1.79	+1.46	+0.33	2.31
32				+2.46	+2.05	+0.41	2.75
29				+2.26	+1.76	+0.51	3.18
4	In chromo-	In 101	Generally	+0.61	+0.52	+0.09	0.58
28	center or		low or	+0.75	+0.63	+0.12	0.97
35	in region		absent	+0.63	+0.59	+0.04	0.22
25*	adjacent to			+0.15*	-0.16*	+0.31*	2.00*
	chromocenter			+0.61*	+0.28*	+0.33*	2.46*
17	In	To the	Generally	+0.39	+0.37	+0.02	0.06
40*	euchromatic	right	low of	+0.99*	+0.61*	+0.38*	2.58*
22	region	of <i>ci</i>	absent	+0.32	+0.26	+0.06	0.50
41				+0.51	+0.57	-0.06	0.37

* Aberrant results, but the individual data are homogeneous.

sively with the same *Rx(ci)/ci ey^R* male and their progeny were raised in the same bottle. At the time of hatching, all the males were classified for their *ey* or *ey⁺* phenotype and mated individually with virgin females to distinguish between fertile (XY) and sterile (X0) males. The four genotypes were then classified for interruption of the fourth vein. Under these conditions, it is possible to compare directly the X0 and XY position effects, without going through the estimation of the expressivity index. The significance of the results may then be tested by the chi-square method. In these cases, the results of the tests were in good agreement with those obtained with the general method.

EXPERIMENTAL RESULTS

The *R(ci)* 2;4 and 3;4 translocations belong to different classes, as mentioned previously. Tables 2 and 3 summarise the data for all the translocations tested, giving the expressivity index for X0 and XY males and the deviation between these two values with the corresponding t values. These data have been obtained from one or many series of experiments as described in the preceding chapter.

TABLE 3

Influence of the absence of the Y chromosome on position effect of the 2;4, R(ci)/ci translocations

<i>R(ci)</i> No.	Break in chromosome		Position effect	Expressivity index		$(d_0 - d_Y)$	t-value
	2R	4		X0 $=d_0$	XY $=d_Y$		
36	In	To the	Generally	-0.10	-0.47	+0.37	1.86
	euchromatic	right	low but	+0.25*	+0.10*	+0.15*	1.66*
45	region	of <i>ci</i>	negative	-0.56	-0.60	+0.04	0.20
				-0.13*	-0.18*	+0.05*	0.61*

* Results obtained using lines of other origin (see text).

TABLE 4

Expression of the ci phenotype in R23(ci)/ci ey, X0 and XY males (first series)

Genotype	Culture No.	<i>ci ey/ci ey</i>							<i>R23(ci)/ci ey</i>							Expressivity index		
		0	1	2	3	4	Total	<i>m</i>	σ^2	1	2	3	4	5	Total		<i>m</i>	σ^2
X0 ♂	636	.	6	5	2	.	13	1.70	...	1	1	1	5	4	12	3.83	...	2.13
	639	1	2	7	1	.	11	1.73	...	.	4	1	7	15	27	4.22	...	2.49
	641	.	6	14	4	1	25	2.00	...	.	.	2	12	10	24	4.33	...	2.33
	643	.	.	5	3	1	9	2.56	...	.	.	2	13	4	19	4.11	...	1.55
	645	2	6	16	2	.	26	1.69	...	.	3	4	12	13	32	4.09	...	2.40
XY ♂		3	20	47	12	2	84	1.88	0.61	1	8	10	49	46	114	4.15	0.83	2.27
	636b	.	1	6	2	.	9	2.11	...	1	1	3	7	3	15	3.67	...	1.56
	639b	.	3	4	1	.	8	1.75	...	.	1	.	5	3	9	4.11	...	2.36
	641b	.	4	12	3	.	19	1.95	...	.	.	2	10	6	18	4.22	...	2.27
	643b	.	3	13	6	2	24	2.29	...	.	2	3	15	11	31	4.12	...	1.83
	645b	.	2	10	5	.	17	2.18	...	.	3	5	4	7	19	3.79	...	1.61
		.	13	45	17	2	77	2.10	0.49	1	7	13	41	30	92	4.00	0.87	1.90

Deviation between X0 and XY expressivity indexes: +0.37 $t = 2.12$

Tables 4 and 5 show for translocation *R23(ci)*, details of the treatment of the data.

Influence of the absence of the Y chromosome on the expression of ci in the control ci ey/ci ey: When the mean of interruption of the fourth vein is calculated for different control groups, the values obtained for the X0 and XY genotypes, although very similar, are sometimes shown to be significantly different, by statistical analysis. However, data provided by experiments in which X0 and XY males were raised in the same culture give mean interruption values, respectively of 2.32 and 2.34. This difference is not significant, either tested by the analysis of variance ($t = 0.12$) or by a chi-square homogeneity test ($P > 0.5$).

TABLE 5

Expression of the ci phenotype in R23(ci)/ci ey, X0 and XY males (second series)

Genotype	Culture No.	<i>ci ey/ci ey</i>							<i>R23(ci)/ci ey</i>							Expressivity index	
		0	1	2	3	Total	<i>m</i>	σ^2	1	2	3	4	5	Total	<i>m</i>		σ^2
X0 ♂	932	3	11	13	5	32	1.63	...	1	11	14	8	6	40	3.18	...	1.56
	936	5	6	13	4	28	1.57	...	.	2	15	2	2	21	3.19	...	1.62
	937	2	6	19	3	30	1.77	...	.	8	27	4	.	39	2.90	...	1.13
	945	1	8	13	5	27	1.81	...	2	3	20	5	.	30	2.93	...	1.12
		11	31	58	17	117	1.69	0.70	3	24	76	19	8	130	3.04	0.70	1.35
XY ♂	932b	2	4	12	2	20	1.70	...	2	9	19	.	3	33	2.78	...	1.08
	936b	1	4	4	1	10	1.50	...	.	4	6	.	.	10	2.60	...	1.10
	937b	.	3	7	2	12	1.92	...	.	6	13	.	.	19	2.68	...	0.76
	945b	1	2	10	2	15	1.87	...	1	2	18	3	1	25	3.04	...	1.17
		4	13	33	7	57	1.75	0.61	3	21	56	3	4	87	2.82	0.56	1.07

Deviation between X0 and XY expressivity indexes: +0.28 $t = 1.69$

It could be shown by statistical analysis that the abnormally high difference observed between X0 and XY means in certain groups of experiments was the consequence of cultures with an abnormally high or low *ci* expression. A shift toward an extreme phenotype can produce a skewed distribution owing to the defect of our classification system. In such cases, the difference between X0 and XY males may well be biased.

We may thus conclude that the *ci* phenotype is particularly sensitive to different external factors, but that male control homozygotes are not sensitive to the absence of the Y chromosome.

Influence of the absence of the Y chromosome on the ci position effect: The analysis of the data reported in Tables 2 and 3 shows that the influence of the absence of the Y chromosome upon the *ci* position effect is related, in the majority of the cases, to the type of translocation and to the intensity of the accompanying position effect.

The *strong position effects* associated with the first class of translocations listed in Table 2 are *modified by the absence of the Y chromosome*. This class represents the *Dubinín effect* produced when *ci* is shifted to euchromatin of an autosome, here the third chromosome. The stock *R31(ci)*, which gives a negative position effect will be dealt with later. All other rearrangements of this first type give a strong positive position effect and show an important deviation between X0 and XY expressivity indexes with a significant or at least a high *t* value. This deviation is positive for all the translocations except for *R5(ci)*, where it is negative. This opposite reaction has been regularly observed in all individual cultures of this line. It is to be noted that the XY male expressivity index in our experiments is higher than the one described by STERN and KODANI (1.01 and 0.22 respectively). The possibility of a spontaneous modification of this strain cannot be excluded, but has not been tested.

The *R52(ci)* translocation described by STERN and KODANI (1955) as a member of this same class (break in chromosome 3L immediately before 76A1; break in chromosome 4 in 101F) has also been tested, though it is not listed in Table 2. Genetical and cytological data obtained after the present work was completed showed that the *ci* locus was no longer linked to the third but to the fourth chromosome. The phenotypic expression of *R52(ci)* homozygotes at low temperature was curiously similar to that of the *ci* allele *ci^w* (*ci* Wallace) and a contamination with this stock cannot be excluded. Nevertheless, this strain shows a clearly significant reaction to the absence of the Y chromosome, similar to the one observed for the translocations of the present class.

When the position effect is low and positive, e.g. when *ci* is placed in or near the chromocenter—or when a euchromatic region of the third chromosome is placed immediately to the right of the *ci* locus—*absence of the Y chromosome has generally no significant modifying effect* (Table 2—second and third class). In each class, one exception out of four cases gives, however, a significant deviation. All individual results obtained with these exceptional strains *R25(ci)* and *R40(ci)* are homogeneous, and the XY expressivity indexes are in agreement with

descriptions given by STERN and KODANI (1955). There is no indication of a modification of the stocks, but no test has been conducted.

Some translocations give a *negative position effect*. According to STERN and KODANI (1955), translocation *R31(ci)* results from an exchange between the distal part of chromosome 4R containing *ci* and the distal segment of chromosome 3L (see Table 1), and should give a strong positive position effect. However, we have consistently observed a strong but negative position effect. Metaphase plates and salivary chromosome squashes gave no indications suggesting a modification of this rearrangement. *Absence of the Y chromosome makes the phenotype still more normal, thus enhancing the negative position effect significantly.*

The influence of the Y chromosome on the negative position effect of *R31(ci)* was compared with its influence on the *2;4 translocations with negative position effects*, *R36(ci)* and *R45(ci)*. Crosses were first made with our usual *ci ey* and $\bar{y}; ci ey$ strains. We found slightly stronger negative position effects than those described by STERN and KODANI (1955). Moreover, the individual results were abnormally variable. Suspecting the presence of some genic heterogeneity that may have arisen in our stocks, we repeated the crosses with *ci ey* and $\bar{y}; ci ey$ strains newly obtained from the Berkeley laboratory and having the same isogenic origin as our own lines. The results of these experiments are marked by asterisks (Table 3). Individual results were more regular but the negative position effect has disappeared (*R36*) or is strongly reduced (*R45*). Nevertheless, the effect of the Y chromosome is still similar, as can be seen from the values of the deviations between the expressivity indexes.

Considering the X0 condition, it is clear that these translocations do not react like the *R31(ci)* rearrangement: *there is no significant influence of the Y chromosome on the position effect of the R36 and R45 rearrangements.*

DISCUSSION

We shall consider first the results obtained with translocations which through their euchromatic-heterochromatic rearrangements produce a strong positive position effect, a type of Dubinin effect.

With one exception, all cases studied respond to the absence of the Y chromosome. Thus, our results support the opinion that there is no basic difference between the variegated-type position effect and the Dubinin effect. However, the modification which is caused by the absence of the Y chromosome consists usually (in three cases out of four) of a significant exaggeration of the position effect (*R23*, 29, 32). Yet, the *ci* Dubinin effect is best compared to variegation of *lt*, a gene also located in immediate proximity of centromeric heterochromatin. SCHULTZ (1936) showed that, contrary to what happens in the case of variegation for euchromatic genes, the addition of an extra Y chromosome to the genotype enhances the expression of the *lt* position effect, while removal of the Y as in X0 males produces normal eyes. Thus, the reaction which we observe usually does not fit the facts described for *lt*. On the contrary, our data agree with the obser-

vations published by GRELL (1959). The author found that in the case of two 3;4 translocations of the $R(ci^+)/ci$ type the addition of a supernumerary Y to the female genotype reduces significantly the Dubinin effect. The presence of two extra Y chromosomes further enhances this reduction. It was concluded that the effect observed in the case of light cannot be generalized for other variegated-type position effects of heterochromatic genes.

However, among the translocations of this first category, the $R5(ci)$ strain is characterized by a significant reduction of the position effect. It thus behaves like the lt gene.

Weak but positive position effects produced by $R(ci)$'s cytologically different from those described above, usually do not respond significantly to the absence of a Y chromosome. These rearrangements produce less drastic modifications in the immediate vicinity of the ci gene, since they result from hetero-heterochromatic or eu-euchromatic fusions. It is to be expected that in this case the function of this gene is little affected and that it works like the nondisplaced ci gene. The phenotype of this ci gene, as is borne out by our results, remains unaffected by the absence of the Y chromosome. This agrees also with the work of GRELL (1959) on ci homozygous females with XX and XXY constitution. However, two out of the eight investigated translocations of this type differ insofar that there is a significant exaggeration of the position effect in the absence of the Y chromosome. Since there is no indication which casts doubt on the constitution of these two translocations, their behavior suggests a modification of the type of activity of the displaced gene even though the XY phenotype is only weakly affected.

The presence of a 3;4 hetero-euchromatic translocation could be shown in the $R31(ci)$ strain. The nature of the change which has transformed the former positive position effect of this strain to a negative one is unknown. But the great susceptibility of this strain to the absence of the Y chromosome is in agreement with its cytological constitution and with the importance of its position effect, even though the latter is negative. The absence of the Y chromosome is expressed by an exaggeration of the negative position effect, i.e. by the appearance of an even more normal phenotype. Two translocations $R36(ci)$ and $R54(ci)$ whose negative position effect is associated with a special type of 2;4 rearrangement did not manifest significant response to the suppression of the Y chromosome.

If a generalized conclusion can be drawn from these findings, i.e. that the Dubinin effect manifested by certain $R(ci)$ translocations is usually influenced by the suppression of the Y chromosome in the male genotype while rearrangements of other cytological constitutions remain unaffected, it is also true that in each category of rearrangements there are some exceptions to the rule. If these exceptions are not related to a modification of the chromosome rearrangement—which the persistence of the rearrangement previously described does not necessarily prove—then we must admit that the way in which we can define the nature of the reorganization in the vicinity of the heterochromatic gene is not precise enough to determine the importance and the direction of the effect produced by change in the dosage of the heterochromatin of the nucleus. Already in the case of the variegated-type position effect, several authors have pointed out that it is

difficult to discover precise rules to the production and expression of this position effect (PANSIN 1936; KAUFMANN 1942) or to the extension of the spreading effect (DEMEREK 1941). In these cases, heterochromatin plays an important role and it seems that there must be specific differences between various regions of the heterochromatin.

Reports have been published according to which changing the dosage of heterochromatin in the nucleus modifies the expression of many other genetic mechanisms: expression of certain quantitative characters (MATHER 1941), size of cells and ommatidia (BARIGOZZI 1951) frequency of crossing over (SCHULTZ and REDFIELD 1951), chromosome conjugation (GERSCH 1959), expression of podoptera phenotype (GOLDSCHMIDT 1955), mutation frequency in males (KERSCHNER 1949).

That heterochromatin influences the expression of genetic mechanisms as different as those we have enumerated, implies that this substance must play a role in early and essential mechanisms of cellular metabolism. GOLDSCHMIDT long ago called attention in this direction. The fact that heterochromatin is not genetically inert and that the nucleolus is associated with heterochromatin has led SCHULTZ, from 1936, to search for a connection between the action of this constituent and the general nucleic acid metabolism in the cell. In a review, SCHULTZ (1956) recalled cases in which a correlation has been demonstrated between heterochromatin and nucleic acid metabolism. We can add to these facts results that have been obtained with Minute strains (ALTORFER 1953). The slowing down of the development and the reduction in body size of Minutes could suggest a lower nucleic acid metabolism, connected with the synthesis of proteins. That is effectively what was observed. In two strains of different origin, M2x (locus unknown) and M2S7, the quantity of total RNA compared to the total nitrogen is lower in Minute individuals compared to their non-Minute siblings. Moreover, the same alteration is produced by two different loci which may suggest that those regions have similar metabolic functions.

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SUMMARY

The effect of the absence of the Y chromosome in males on the position effect produced by translocations of the *ci* gene has been studied in a series of 16 2;4 and 3;4 *R(ci)* rearrangements. When there is a marked position effect, or Dubinin effect, resulting from a hetero-euchromatic fusion in the vicinity of *ci*, absence of the Y chromosome generally causes a significant modification of the effect, most often enhancing the expression of the translocated *ci* gene. When the position effect is low or zero, the absence of the Y chromosome usually has no influence on the expression of the *ci* character. However, in both cases, some translocations show a statistically significant modification of the *ci* phenotype, opposite to that of the group to which they belong. Two 2;4 translocations, chosen for their nega-

tive position effect, do not show a significant reaction. On the other hand, it has been proved that the control *ci* phenotype is unaffected by the absence of the Y chromosome.—The fact that the Dubinin position effect may be affected by a change in the heterochromatic constitution of the nucleus, allows us to consider it as a form of variegated-type position effect.

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