STUDIES ON THE POSITION EFFECT AT THE CUBITUS INTERRUPTUS LOCUS OF *DROSOPHILA MELANOGASTER*¹

CURT STERN AND MASUO KODANI²

Departments of Zoology, University of Rochester and University of California

Received August 16, 1954

WHEN DUBININ and SIDOROV discovered, in 1934, that translocations affecting the small fourth chromosome of *Drosophila melanogaster* frequently show a changed effect of the normal allele of the cubitus interruptus gene (*ci*⁺), they posed the problem why some rearrangements gave this position effect and why others did not. Answers were provided in a series of papers by DUBININ, SOKOLOV, and TINIAKOV (1935), KHVOSTOVA and GAVRILOVA (1935), PANSIN (1936, 1938) and particularly in the remarkably extensive work of KHVOSTOVA (1936, 1939). The following main conclusions in regard to chromosomes 2, 3, and 4 were reached:

- (a) A necessary condition for a position effect of the *ci* locus is that the break in chromosome 4 lies between the kinetochore and the locus of *ci* (that is "proximal" to *ci*).
- (b) Only those breaks give a position effect which transfer the distal part of the right arm of chromosome 4 to regions of chromosome 2 or 3 distal to the proximal first fifth or quarter of the chromosome arm involved in the translocation.

These investigations involved fourth chromosomes in which a *normal* allele of *ci* had been present at the time the rearrangements were produced. Later, SIDOROV (1941) and STERN and HEIDENTHAL (1944) reported on position effects of the *mutant* *ci* allele. On the basis of evidence from selected rearrangements SIDOROV concluded that the relations regarding the point of breakage in chromosomes 2, 3, and 4, and the existence or nonexistence of a position effect which had been found for *ci*⁺ rearrangements ("*R*(+)" held also for rearrangements involving *ci* ("*R*(*ci*)"). Our own unselected sample of *R*(*ci*) stocks permitted us to detect position effects not accessible to SIDOROV. The present paper will provide data to reconsider the following questions:

- (1) What types of rearrangements give a position effect at the *ci* locus?
- (2) What relation, if any, exists between the kind of rearrangement and the degree of position effect?

In addition experiments on the relative degrees of position effects of *R*(*ci*) "position alleles" in heterozygotes with *ci*⁺ and *ci* alleles, and the phenotypic effects of *R*(*ci*) alleles in homozygotes, hemizygotes and compounds will be presented. Some new data on *R*(+) position effects are also included.

The work covered in this report extended over a long period. The genetic tests were carried out first at the University of Rochester and later at the University of

¹ Dedicated to Professor Alfred Kühn, Tübingen, on the occasion of his seventieth birthday.

² Present addresses: University of California, Berkeley, California, and Atomic Bomb Casualty Commission, American Embassy, Tokyo, Japan, respectively.

California by DRs. GERTRUDE HEIDENTHAL, H. D. STALKER, ELIZABETH WHITE SCHAEFFER, R. H. MACKNIGHT, M. KODANI, ALOHA HANNAH, C. STERN and MISS JOSEPHINE DE FRANK. Most of the cytological analyses are due to M. KODANI though H. D. STALKER had made a series of preliminary determinations and ALOHA HANNAH has, more recently, checked certain critical points. The names of all investigators could well have been listed as co-authors.

MATERIALS AND METHODS

The bases of investigation were 55 $R(ci)$ rearrangements from irradiated $ci\ ey^R$ males (ey^R = eyeless R , a recessive mutant in chromosome 4) and 19 $R(+)$ rearrangements derived from X-rayed wild type Canton-S males. The $R(+)$ rearrangements were detected by means of a ci position effect (STERN, SCHAEFFER, and HEIDENTHAL 1946). The $R(ci)$ rearrangements were found, without reference to any possible position effect, by their giving linkage data characteristic of translocations involving chromosome 4 (STERN and HEIDENTHAL 1944).

All $R(ci)$ alleles were originally derived from Y-bearing sperm. Among them one case of a Y-4 translocation was found, $R^1(ci)$, and two complex translocations, $R^{12}(ci)$ and $R^{40}(ci)$, involving the Y chromosome simultaneously with 2-4 or 3-4 rearrangements ($T(Y; 2; 4)$ and $T(Y; 3; 4)$). $R^1(ci)$ will not be considered in this report. When the tests for position effects were made $R^{40}(ci)$ had, presumably as a result of crossing over, become free of the Y-translocation but had retained a simple 3-4 translocation. A special rearrangement $R^{24}(ci)$, which involves a duplicate insertion of a section of 4 into 3, will not be treated in this paper. Finally 10 $R(ci)$ stocks were lost before completion of both cytological and genetic studies.

The $R(+)$ alleles originally came from either X- or Y-sperm. Among the 17 $R(+)$ alleles for which both genetic and cytological tests are available no X-4 or Y-4 rearrangement was present. Two stocks of $R(+)$ alleles were lost before completion of the analysis.

The following genetically different types of chromosome 4 were used in various crosses: ci^{+C} , the wild type allele of ci present in the Canton-Special stock; $ci^{+2}\ ey^2$, eyeless², recessive, and the wild type allele of ci^{+2} present in the ey^2 stock (STERN and SCHAEFFER 1943b); ci , cubitus interruptus; $ci\ ey^R$, cubitus interruptus, eyeless^R; ey^D , eyeless-dominant; $M-4$, Minute-4, a deficiency of several bands in region 101F, all of 102A and including 102B3; $M-4^b$, another deficiency, involving the right arm of chromosome 4 from close to the centromere to immediately after 102C4 (listed as $M-4(+)-15$ by FUNG and STERN 1951). Apart from chromosome 4 which undergoes very little crossing over, all stocks used either in furnishing the rearrangements or in crosses had been made relatively isogenic with each other before the beginning of this work.

As reported in earlier papers the degree of interruption of veins was estimated by arbitrarily distinguishing five classes—0, 1, 2, 3, 4—from no interruption to complete absence of the section of the fourth vein located between the posterior cross vein and the wing margin (see fig. 1; STERN, MACKNIGHT, and KODANI 1946). A mean value of expressivity was then calculated by giving each class the weight of its class designation 0-4 and determining the mean of the distribution. Separate means

FIGURE 1. Representative samples of wings showing from no interruption (class 0) to complete absence (class 4) of the cubital vein.

were calculated for each culture, and each genotype and sex. In any one experiment total mean values for each genotype and sex were determined from the sum of the distributions of all cultures. This method of obtaining mean values of expressivity is admittedly crude. A better determination of means, particularly in cases of presumably truncated distributions is offered by transforming the data to inverse probability distributions. This has recently been done by HOUSE (1953) in a study of developmental interaction of mutants affecting venation. However, for the purposes of the present paper it seemed sufficient to employ the mean values of expressivity as derived in the simpler fashion. Various uncertainties are attached to the determination of mean expressivity of position effect alleles, as will be seen later. Therefore this account will be concerned with general trends rather than with specific values whose inherent uncertainties would be covered up by the pretense of exactness which is implied in the use of more refined statistical constants. In general the mean expressivity of any given genotype involving $R-$ alleles will not be reported as such but rather in relation to a similar genotype without an $R-$ allele. Thus, for instance, the mean expressivity value of $R(ci)/ci$ individuals will be compared to that of ci/ci sibs and the *difference* between the two values, designated as expressivity index, entered in the tables. A positive difference indicates a higher expressivity of the $R-$ genotype, a negative difference a lower expressivity.

Cytologically, rearrangements were analysed by means of permanent acetocarmine and aceto-orcein preparations of salivary gland chromosomes, and in reference to BRIDGES' map (1935a). Double bands were counted as units so that, for instance, the band 102B1 often referred to below actually corresponds to 102B1.2 of later maps (SLIZYNSKY 1944). For special purposes mitotic figures from neuroblasts and oogonia, were also studied.

THE LOCUS OF *CI*

The first data regarding the position of the *ci* locus within a specific region of chromosome 4 were provided by BRIDGES (1935b) by means of the $M-4$ deficiency. $M-4/ci$ flies exhibit an exaggerated *ci* phenotype. This pseudodominance of *ci* was interpreted to mean that the deficient region, from 101F to and including 102B3 had contained the *ci* locus (fig. 2). At the same time it was found by DUBININ, SOKOLOV, and TINIAKOV (1935) and confirmed by KHVOSTOVA and GAVRILOVA (1935), PANSHIN (1936), and KHVOSTOVA (1936) that a position effect of a normal allele of cubitus

FIGURE 2. Map of salivary chromosome 4 of *D. melanogaster* combined after BRIDGES (1935 a, b) and SLIZYNSKI (1944) with locations of breakage points in various duplications, deficiencies and rearrangements. The two solid arrows indicate limiting breaks with heavy or weak position effects, respectively, of $R(ci)/+$.

interruptus appeared only when the break in chromosome 4 was in the immediate neighborhood of the centromere, or more specifically to the left of or within region 101F of the salivary chromosome map. From this it was concluded that the locus of *ci* lies to the right of 101F, and that a position effect is caused by the removal of the ci^+ allele from the heterochromatin in the centromere region.

Further evidence for localization of the *ci* locus in the right arm of the fourth chromosome is provided by three rearrangements in which the phenotypes of flies with the translocated part of chromosome 4 but without the remainder of the broken chromosome 4 showed the presence of the *ci* locus: (1) In $R^{21}(+)$, with breakage points in chromosome 2 just after 60E1 and in chromosome 4 in 101F, individuals deficient for the tip of 2 and having the translocated distal fragment of 4 in addition to a normal chromosome 4 with *ci* show a shift of venation toward normal thus indicating possession of the $R(+)$ allele in the fragment; (2) In $R^{15}(+)$ with an insertion into 30A of chromosome 2 of a section of chromosome 4 extending from immediately before 101F1 to immediately after 102C4, individuals which possess the insertion in addition to two cytologically normal chromosomes 4 with *ci* likewise show by a shift toward normal venation that the insertion contains the $R(+)$ allele; (3) Similarly, in $R^{24}(ci)$ the insertion into chromosome 3, just before 93F1 of a duplicated, inverse tandem section from close to the kinetochore of chromosome 4 up to and including 102D1 leads to a shift toward normal venation in flies possessing two cytologically normal chromosomes 4 with *ci*, thus placing the $R(ci)$ allele in the insertion. Furthermore, $R^{24}(ci)$ derivatives which have normal chromosomes 3, one *ci*-containing normal chromosome 4 and the deleted chromosome 4, show by their extreme, hemizygous-like vein interruption that the deleted chromosome does not contain the *ci* locus.

Indirect evidence on the localisation of the *ci* locus is based on the degree of position effects produced by breakages in different regions of chromosome 4. DUBININ and his collaborators observed position effect of *R*(+) alleles only when the break in 4 occurred in its right arm to the left of the *ci* locus. Cytologically, these breaks are close to the centromere, in section 101 or 102A. Thus, *ci* is located in the range 102B to 102C4. The cytology of our 17 *R*(+) alleles agrees with this conclusion. In 13 cases the break in 4 had taken place in the right arm of chromosome 4 in regions 101D-F, in three cases immediately before the band 102B1, and in one case in the left arm of 4.

It will be shown that position effects of *R*(*ci*) alleles occur not only with breaks to the left of 102B1 but also with breaks to the right of this band. However there is a striking difference in degree of effect according to whether the break lies to the left or the right of 102B1. In the 15 translocations involving the distal regions of chromosome 2 or 3 with a break in chromosome 4 to the left of 102B1 the index of expressivity of *R*(*ci*) alleles in combination with + (= *R*(*ci*)/+) ranged for females from .73 to 2.49, and for males from .85 to 2.62 (table 1). On the other hand the corresponding indexes for the 13 *R*(*ci*) alleles involving distal regions of chromosomes 2 or 3 with the fourth chromosome break to the right of 102B1 ranged for the females from 0 to only .14 and for the males from -.05 to only .20 (table 3).

Among the *R*(*ci*) alleles with a break to the left of 102B1, and with correspondingly high expressivity in *R*(*ci*)/+ there were three in which the break was located rather close to 102B1. In *R*³²(*ci*) it had occurred just before 102A1 and in both *R*⁵(*ci*) and *R*¹⁸(*ci*) it immediately preceded 102B1.

Among the *R*(*ci*) alleles with a break to the right of 102B1, and with correspondingly low expressivity in *R*(*ci*)/+ there were four in which the break was located rather close to 102B1. In three of the alleles, *R*¹⁷(*ci*), *R*³⁶(*ci*) and *R*⁴¹(*ci*) the break was just before 102C1, and in *R*²²(*ci*) it was immediately after 102B1. These facts narrow down the region for which a rearrangement to the left of it causes a strong position effect and one to the right of it a weak effect. Altogether the findings show the band 102B1 or its immediate neighborhood to be of crucial importance.

The classical interpretation of such results assigns the locus of *ci* to the band 102B1 or its immediate neighborhood. It has, however, been pointed out that the existence of a position effect may weaken the validity of a sharp definition of a locus (for a detailed discussion see GOLDSCHMIDT 1946). The data reported above, while showing effects of breaks occurring over a considerable length, are at least fully compatible with an assignment of the *ci* locus within or directly adjacent to the 102B1 band.

The question may be raised whether there is, typically, only one locus of *ci* or whether there may be a sequence of closely linked *ci* sites similar to the cases of pseudo-allelism discovered by E. B. LEWIS (1945), GREEN and GREEN (1949) and others. No data bearing on this question are available. At present the assumption of a single locus, at or close to band 102B1 appears sufficient.

THE EFFECTS OF *R*(*CI*) ALLELES HETEROZYGOUS WITH *CI* OR +

The position effect of the *R*(*ci*) alleles in heterozygotes was measured in two ways, by comparing the average vein interruption of *R*(*ci*)/*ci* with that of *ci*/*ci* flies,

and of $R(ci)/+$ with that of $ci/+$. Since various environmental agents affect the degree of interruption in ci and $R(ci)$ genotypes, the interruptions of $R(ci)/ci$ and $R(ci)/+$ flies were not compared with a standard value for ci/ci and $ci/+$ but with values obtained from sibs of the $R(ci)$ individuals. For this purpose two types of crosses were made with all $R(ci)$ alleles, usually $ci\ ey^R \text{ } \varnothing \varnothing \times R(ci)\ ey^+/ci\ ey^R \text{ } \sigma^7 \sigma^7$ and $ci^{+2}\ ey^2 \text{ } \varnothing \varnothing \times R(ci)\ ey^+/ci\ ey^R \text{ } \sigma^7 \sigma^7$. The F_1 of these crosses consisted of the two types of flies to be compared which had developed under essentially identical conditions. This method of comparison had the further advantage of largely cancelling the effects of possible modifiers of vein interruption which may have arisen in any one of the $R(ci)$ stocks.

On the other hand two possible sources of error are introduced in these crosses. One consists in the fact that some of the $R(ci)$ stocks contained individuals with two normal fourth chromosomes besides the $R(ci)$ translocation. This may lead to both diplo- and triplo-4 offspring. Values for degree of position effect derived from such mixed groups are not strictly comparable with values from diplo-4 flies only. This source of variation will be discussed in connection with selected, critical cases. For the majority of the results no detailed information on the diplo-4 or triplo-4 status of the flies is available. Presumably it accounts for a part of the variation in the observed values for position effects.

The second possible error in the determination of the effects of $R(ci)$ alleles lies in the fact that the $R(ci)$ individuals are non-eyeless (heterozygous for ey^R or ey^2) while their sibs are homozygous eyeless. However, homozygosity for ey^R or ey^2 has no appreciable influence on the expressivity of vein interruption. In crosses of $ci\ ey^R \text{ } \varnothing \varnothing \times ci\ +/ci\ ey^R \text{ } \sigma^7 \sigma^7$ the mean interruption in 390 $ci\ +/ci\ ey^R$ daughters was 2.65 as compared to 2.64 in 323 $ci\ ey^R$ sisters, and 2.92 in 376 $ci\ +/ci\ ey^R$ sons as compared to 2.97 in 434 $ci\ ey^R$ brothers. The differences between the means for each sex, .01 and -.05, are not significant. Similarly, in two sets of crosses of $ci^{+2}\ ey^2 \text{ } \varnothing \varnothing \times ci\ +/ci\ ey^R \text{ } \sigma^7 \sigma^7$ (17°C) no significant differences in expressivity of $ci\ +/+ \text{ } ey^2$ and $ci\ ey^R/+ \text{ } ey^2$ sibs existed. In the first experiment mean vein interruptions in 2988 non-eyeless and 2463 eyeless females were .08 and .07, respectively, and, in 2793 non-eyeless and 2448 eyeless males, .13 and .11. The differences thus were .01 and .02. In the second experiment, 305 non-eyeless and 160 eyeless females had mean interruptions of .01 and .02, and 270 non-eyeless and 194 eyeless males .04 and .06. The differences, -.01 and -.02 are not significant. Since the eyeless genotype does not affect the expressivity of ci , references to the ey alleles will be omitted in the following except for table headings.

As seen in the foregoing, the expressivity of alleles at the ci locus is different in the two sexes. This is the reason why all data will be given separately for females and males. There is also a difference in the temperature at which various types of experiments were carried out. Low temperature increases the penetrance and mean expressivity of ci alleles. Therefore, low temperature was used in combinations in which penetrance at high temperature is close to zero. Unless otherwise noted all cultures were reared at $\pm 26^\circ\text{C}$ except those with $R(ci)+$ which were kept at $\pm 17^\circ\text{C}$.

Translocations of ci to a distal region of chromosomes 2 or 3

Table 1 gives data on the 15 translocations in which a break in chromosome 4 had occurred to the left of band 102B1 and a break in chromosome 2 or 3 in any region except 37-44 and 77-85. These regions, located proximally on both sides of the centromeres 2 and 3 had been found by KHVOSTOVA (1939) not to produce position effects with *ci*. The translocations are listed in order of region to which the translocated part of 4 became attached. All translocations are simple reciprocals except $R'(ci)$ in which the distal section of 4 is translocated to 24A1, of 2L, the tip of 2L substituted for the tip of 3R beyond 98A1 and the tip of 3R translocated to the base of 4. In addition the section 27B-29B is inverted.

These 15 $R(ci)$ alleles show highly significant position effects. $R(ci)/ci$ females are from .59 to 1.70 and $R(ci)/ci$ males from .22 to 1.63 class values more extreme than their like sexed sibs. Even more striking are the differences between $R(ci)/+$ and $ci/+$ flies, which range from .73 to 2.49 for females and from .85 to 2.62 for males. Since most $ci/+$ flies even at 17°C are normal—the mean expressivity being below .1—the indexes for $R(ci)/+$ flies in table 1 are an expression of the fact that usually the majority and sometimes all individuals show vein interruptions.

The variability of the degree of position effects in the different $R(ci)$ alleles will be discussed later.

TABLE 1

The effects of translocations of ci to a distal region of chromosomes 2 or 3. F_1 of $ci\ ey^R\ ♀♀ \times R(ci) +/ci\ ey^R\ ♂♂$ and $+ey^2\ ♀♀ \times R(ci) +/ci\ ey^R\ ♂♂$

Number of $R(ci)$	Break in chromosomes**		Index $R(ci) +/ci\ ey^R$		N†	Index $R(ci) +/+ ey^2$		N‡
	4*	2 or 3	♀♀	♂♂		♀♀	♂♂	
7	101	2L i. b. 24A1	1.64	1.22	137	.73	1.07	127
53	101	2R i. a. 47B5	.92	.74	1760	1.47	1.60	389
33	101F	2R betw. 48C & D	.89	.80	333	1.58	1.73	281
3	101	2R i. b. 52E1	1.04	.79	118	1.40	1.49	317
55	101F	3L i. a. 65F2	.76	.64	1231	.99	1.13	275
5	i. b. 102B1	3L a. 69E2	.62	.22	456	.78	.85	374
8	101	3L a. 69F2	1.23	1.63	97	1.96	1.93	604
31	101	3L i. b. 75A1	.77	.87	260	2.26	2.46	293
52	101F	3L i. b. 76A1	1.70	1.28	1021	1.44	1.63	827
18	i. b. 102B1	3R a. 86C8	1.08	.94	1394	2.49	2.62	613
54	101F	3R 87F	1.29	1.09	435	—	—	—
23	101F	3R a. 92E2	.59	.29	1395	2.10	1.98	464
32	i. b. 102A	3R i. a. 92E2	1.04	.95	144	2.01	1.97	401
29	101	3R i. a. 94B2	1.34	1.04	1063	2.33	2.39	316
19	101	3R i. a. 97D9	1.26	.98	232	.92	1.12	279

* 101 signifies a break to the right of the centromere and to the left of region 101F.

** i. b., i. a. = immediately before or after . . .

† Sum of $R(ci) +/ci\ ey^R$ and $ci\ ey^R/ci\ ey^R$.

‡ Sum of $R(ci) +/+ ey^2$ and $ci\ ey^R/+ ey^2$.

Translocations of ci to a proximal region of chromosomes 2 or 3

Among the translocations with a break to the left of 102B1 there were five which had their only other break close to, and six which had it within the chromocenter of chromosomes 2 or 3 (table 2). None had multiple breaks. In contrast to the preceding group of $R(ci)$ alleles the position effects of the present group are much less striking or even absent.

Postponing briefly a consideration of $R^9(ci)$ and $R^{42}(ci)$ it is noted that the position effect indexes for the translocations with breaks slightly distal to the chromocenter of chromosomes 2 or 3 are smaller than the indexes for the translocations with breaks within the chromocenter. In the group with slightly distal breaks 15 values out of a total of 18 are within $\pm .15$ of zero and thus are indicative of absence or near-absence of a position effect. The occurrence of two small negative values among the 15 may be regarded as a chance deviation from 0 or from a small intrinsically positive value. In contrast to the group with slightly distal breaks in chromosome 2 or 3 all 22 values in the group where the break was located directly in the chromocenter of 2 or 3 were larger than $+.15$, most of them considerably so.

$R^9(ci)$ showed very low position effect values in the $R^9(ci)/ci$ heterozygotes but relatively high values in $R^9(ci)/+$. The existence of a somewhat pronounced position effect for $R^9(ci)$ is not unexpected. The break point in chromosome 2, in 38B, is considerably more distant from the centromere than the break points of any other $R(ci)$ alleles listed in table 2. Cytologically, $R^9(ci)$ thus approaches the $R(ci)$ alleles of table 1 and the existence of some relatively high position effect values is the genetic counterpart of its cytological peculiarity.

The position effect values listed in the first of the two tests for $R^{42}(ci)$ are out of

TABLE 2

The effects of translocations of ci to a proximal region of chromosomes 2 or 3. F_1 of crosses as in table 1

Number of $R(ci)$	Break in chromosomes		Index $R(ci) +/ci\ ey^R$		N	Index $R(ci) +/+ \ ey^2$		N
	4	2 or 3	♀ ♀	♂ ♂		♀ ♀	♂ ♂	
9	101	2L a. 38B2	.17	.02	587	.47	.57	583
35	101	3L 79F	.02	— .01	465	.03	0	341
4	101	3L 80	— .12	.09	470	.02	.06	200
34	101	3L 80	.20	.17	1794	.08	.16	1726
34			—	—	—	.07	.15	420
25	101	3R 81F	.15	.06	1404	.11	.07	854
30	101	2 chrc.	.29	.17	357	.18	.20	360
42	101	2 chrc.	(— .16	— .10	946	.02	.01	621)
42			.48	.61	2780	.23	.37	1274
44	101	2 chrc.	.54	.61	489	.34	.58	1177
28	101	3 chrc.	.67	.63	679	.78	.66	282
37	101	3 chrc.	.45	.37	1395	.45	.52	3592
37			—	—	—	.28	.41	394
48	101	3 chrc.	.62	.50	1836	.33	.53	2261

line with the rest of the values for the $R(ci)$ alleles with a break within the chromocenter. The experiments which gave these $R^{42}(ci)$ values were carried out at the time when the main body of $R(ci)$ values were determined. More recently, when the unusual nature of the $R^{42}(ci)$ values was recognized, new experiments with $R^{42}(ci)$ were undertaken and the cytology of the stock re-investigated. The cytological nature of the translocation was confirmed but the genetic results given in the second row were clearly different from the earlier ones. The position effect values were now completely in line with those of the other $R(ci)$ alleles with a chromocentral break in 2 or 3. It is possible that an $R(ci)$ allele other than $R^{42}(ci)$ had mistakenly been used in the first experiment. The position effect values of only the more recent experiment will be considered in the later discussion.

Translocations of distal regions of chromosomes 2 or 3 to ci

In 13 translocations the break in 4 had occurred at various points to the right of 102B1, and the break in 2 or 3 anywhere outside the proximal regions 37-44 and 77-85. None involved 2L, six 2R, only one 3L and six 3R (table 3). All but two of these translocations are simple reciprocals in which the distal part of a long autosome

TABLE 3

Translocations of distal regions of chromosomes 2 or 3 to ci. F_1 of crosses as in table 1

Number of $R(ci)$	Break in chromosomes		Index $R(ci) +/ci\ ey^R$		N	Index $R(ci) +/+ ey^e$		N
	4	2 or 3	♀♀	♂♂		♀♀	♂♂	
16	a. 102D3	2R i. a. 45E1	-.23	-.05	305	0	-.04	352
16			-.10	-.08	1463	—	—	—
12*	102F	2R 46	—	-.30	544	—	0	144
36	i. b. 102C1	2R i. a. 47A1	-.22	-.19	2111	.02	.01	460
36			.02	-.13	176	—	—	—
36			-.12	-.02	2037	—	—	—
6	a. 102D3	2R a. 47D3	-.14	-.13	375	.01	.04	249
10†	i. a. 102C1	2R i. a. 48B1	-.23	-.29	1474	0	.01	256
45	a. 102 D-E	2R 58D	-.61	-.57	1906	.06	-.02	368
45			-.60	-.75	990	.07	.08	1237
45			-.40	-.48	5540	.14	-.05	966
17	i. b. 102C1	3L i. b. 67E1	.12	-.09	189	.03	.04	251
43	102F	3R i. b. 86A1	.10	.08	609	0	0	661
26	i. a. 102D1	3R i. a. 90B1	.31	.19	2982	.03	.02	1017
26			—	—	—	.06	-.01	809
40	102F2	3R i. b. 94A1	.73	.42	574	.13	.20	1143
41	i. b. 102C1	3R 98 C-D	.17	.15	2302	0	0	1983
22	i. a. 102B1	3R i. a. 98C1	.15	.81	998	0	.04	244
15	i. a. 102C1	3R i. a. 100A2	.37	.20	670	.05	.03	384

* Complex cytology: $T(Y; 2; 4)$. No data on females. See text for calculation of $R(ci) +/ci\ ey^R$ index.

† Complex cytology.

had become attached to the base of 4 which had retained the *ci* allele. The two more complex translocations are $R^{10}(ci)$ and $R^{12}(ci)$. In $R^{10}(ci)$ six breaks have been established by observation, and a seventh, in the left arm of 4, is postulated. The established breaks are located (1) immediately after 48B1, (2) immediately before 50A1, (3) between centromere 3 and 81F, (4) in 85F (5) immediately after 97F1, and (6) immediately after 102C1. The new order is as follows: 2L, 2R to 48B1; 85F1-97F; 102C-102F. 3L, 3R to 81F; 50A1-60F. 4L tip; 81F-85F; 4L base, 4R to 102C1; 48B1-49F; 97F-100F. The most important part of this complex as far as it concerns the $R(ci)$ position effect is the chromosome which contains the centromere and the proximal regions of the original chromosome 4. Its left arm contains an intercalated proximal section of 3R and its right arm consists of the proximal part of 4, including the *ci* locus, followed by a short distal segment of 2R, and ending with the tip of 3R.

$R^{12}(ci)$ involves three clearly established and two postulated breaks. The former are all in 2R, at 46, 54B and 57B, the latter in 4 at 102F and in the Y chromosome. The rearrangement is as follows: 2L, 2R to 46; tip of 102F. 4L, 4R to 102F; 46-54B, 57B-60. Y with inserted 54B-57B. In regard to the new association involving the *ci* locus, $R^{12}(ci)$ may be described as a case in which the tip of 4 has been replaced by the right arm of 2R beginning at 46 but excluding a section from 54B to 57B. On account of the Y-linkage involved in this translocation usually no $R^{12}(ci)$ females are produced and, in crosses of $R^{12}(ci)/ci$ males to *ci/ci* or *+/+* females, the *ci/ci* or *ci/+* sons have duplications for the Y-inserted section of 2. Since the *ci/ci* males with the duplication are significantly more extreme in expressivity than normal *ci/ci* males, the position effect of $R^{12}(ci)/ci$ males has been measured not against the mean value of their sibs but against that of the nearly 10,000 *ci/ci* male sibs of other $R(ci)/ci$ flies.

Beginning with a discussion of $R(ci)/ci$ heterozygotes with the T(2R distal; 4 distal) $R(ci)$ alleles, each of the 11 initial indexes for the first tests are negative, that is, the mean degree of vein-interruption in the position allele heterozygote is less than that of *ci* flies with normally arranged chromosomes. One or more recent repeat experiments for three of the alleles, $R^{16}(ci)$, $R^{36}(ci)$ and $R^{45}(ci)$ added 10 more values

TABLE 4

Numbers of cultures for six T(2R distal; 4 distal) R(ci) alleles which gave positive, negative and zero position effect indexes for R(ci)/ci

Number of $R(ci)$	Index ♀♀			Index ♂♂		
	+	—	0	+	—	0
6	1	2	0	2	1	0
10	2	7	0	0	8	1
12	—	—	—	0	6	0
16	4	8	0	5	7	0
36	9	16	0	8	16	1
45	2	56	1	1	58	0
Total	18	89	1	16	96	2

which are listed in the second and third entries for these alleles. Nine of the indexes were again negative, one was positive, but very small (.02). Most of the $R(ci)/ci$ values deviated from ci/ci considerably less than one third of a class interval, but the six values for $R^{45}(ci)/ci$ were from .40 to .75 of a class interval more normal than those of their ci/ci sibs. While some of the negative values do not differ significantly from 0, on the whole the shift toward normality of venation in the six T(2R distal; 4 distal) $R(ci)$ alleles in $R(ci)/ci$ as compared to ci/ci flies is significant. This is reflected in table 4 which gives the number of individual cultures with positive, negative, and 0 differences. Out of the 222 indexes 185 are negative.

The single $R(ci)$ allele with one break to the right of ci and another in 3L, $R^{17}(ci)$, gave a small positive index for $R(ci)/ci$ females and a small negative index for males. These values do not differ significantly from zero, possibly because the numbers of individuals studied were small.

All 12 values for the differences between $R(ci)/ci$ and ci/ci sibs of the six $R(ci)$ alleles with one break to the right of ci and another in 3R were positive but relatively small.

All differences between $R(ci)/+$ and $ci/+$ of the present series of $R(ci)$ alleles are zero or very small. Only four values are negative. It might have been expected that the $R(ci)$ alleles with negative position effects in $R(ci)/ci$ heterozygotes would likewise give negative effects in $R(ci)/+$ heterozygotes. The $R(ci)/+$ experiments, however, were not suitable to revealing such possible effects. Since the mean expressivity for $ci/+$ in any experiment is often zero and since a mean of 0 signifies normality of venation which forms a phenotypic endpoint of the range of expressivity, $R(ci)/+$ flies even if intrinsically more normal than $ci/+$ flies cannot yield a still lower value. Even in experiments in which some $ci/+$ flies show vein interruptions the mean is so close to zero that no significant values for negative position effects can be obtained.

Translocations of regions including the proximal parts of chromosomes 2 or 3 to ci

In four of the $R(ci)$ rearrangements one break had occurred close to or within the chromocentric part of chromosomes 2 or 3 and the other in chromosome 4 to the right of 102B1. In these simple reciprocal translocations most or all of an arm of the long autosome had become attached to chromosome 4 distally to the ci locus. None of the position effects as measured in $R(ci)/ci$ flies was large (table 5). Seven values were

TABLE 5

Translocations of regions including the proximal parts of chromosomes 2 or 3 to ci . F_1 of crosses as in table 1

Number of $R(ci)$	Break in chromosomes		Index $R(ci)/ci$ ey^R		N	Index $R(ci)/++$ ey^2		N
	4	2 or 3	♀♀	♂♂		♀♀	♂♂	
2	a. 102C3	2R i. a. 44C3	.32	— .11	199	0	.02	326
27	i. b. 102F1	3R i. a. 84B3, 4	.28	.18	1669	.02	.14	824
39	i. b. 102D1	3 chrc.	.24	.12	2870	.06	.06	1636
39	—	—	—	—	—	.07	.15	483
13	i. b. 102E1	3 chrc.	.01	.10	709	.03	.08	1078

positive, some of which deviated significantly from 0. One negative value did not differ significantly from 0.

All values for position effects of the $R(ci)/+$ combinations were zero, or positive and very small.

Tests for triplo-4 genotypes

An important source of error in determining position effect indexes would be the presence of any appreciable number of triplo-4 individuals among the $R(ci)$ flies. If, for instance, in the experimental series $ci/ci \text{ } \varnothing \text{ } \varnothing \times R(ci)/ci \text{ } \sigma \text{ } \sigma$ some or all of the P males were actually $R(ci)/ci/ci$ then the F_1 would not consist solely of $R(ci)/ci$ and ci/ci flies but would also contain $R(ci)/ci/ci$ and $ci/ci/ci$ flies. Correspondingly in matings of $+/+ \text{ } \varnothing \text{ } \varnothing \times R(ci)/ci/ci \text{ } \sigma \text{ } \sigma$ the F_1 would consist of four genotypes, namely $R(ci)/+$, $ci/+$, $R(ci)/ci/+$ and $ci/ci/+$. Dosage and interference effects (STERN 1943, 1948) would shift the phenotype of $ci/ci/ci$ toward normal as compared with ci/ci , and of $ci/ci/+$ toward greater abnormality as compared with $ci/+$. Similar shifts, in one or the other direction, would be expected in $R(ci)/ci/ci$ vs. $R(ci)/ci$ and in $R(ci)/ci/+$ vs. $R(ci)/+$ flies.

Triplo-4 individuals do indeed occur in $R(ci)$ stocks as proven by cytologic and genetic tests. It is, therefore, necessary to consider in some detail the consequences of their presence on the position effect indexes reported in tables 1, 2, 3, and 5.

1. $ci/ci \text{ } \varnothing \times R(ci)/ci \text{ } \sigma$ or $R(ci)/ci/ci \text{ } \sigma$

(a) If the $R(ci)$ allele did not exhibit a position effect then $R(ci)/ci/ci$ flies would be phenotypically equivalent to $ci/ci/ci$ and $R(ci)/ci$ to ci/ci . A diplo-4 $R(ci)/ci$ P- σ might produce some $R(ci)/ci/ci$ offspring besides the regular $R(ci)/ci$. Since, barring a position effect, the former would be more normal in venation than the latter, the sum of these $R(ci)$ flies would average more normal than their ci/ci sibs. Therefore, a *negative* value would be obtained for the mean difference between $R(ci)$ and ci flies.

(b) A *negative* value would also be expected if the P σ itself had been triplo-4 $R(ci)/ci/ci$. In this case, the majority of the $R(ci)$ offspring would be $R(ci)/ci/ci$ and the rest $R(ci)/ci$, and the majority of the ci offspring ci/ci and the rest $ci/ci/ci$. Therefore, again barring a position effect, the sum of the $R(ci)$ flies would average closer to normality than the sum of the ci flies.

2. $+/+ \text{ } \varnothing \times R(ci)/ci \text{ } \sigma$ or $R(ci)/ci/ci \text{ } \sigma$

(a) If the $R(ci)$ allele did not exhibit a position effect and the P σ was diplo-4 $R(ci)/ci$ the sum of the regular $R(ci)/+$ and of triplo-4 $R(ci)/ci/+$ F_1 would be less normal than that of the $ci/+$ sibs. Thus, a *positive* difference between the averages of $R(ci)$ and ci flies would be observed.

(b) A *positive* value would also be found if the P σ had been $R(ci)/ci/ci$. In this case, the majority of the $R(ci)$ offspring would be $R(ci)/ci/+$ and the rest $R(ci)/+$ and the majority of the other sibs $ci/+$ and the rest $ci/ci/+$.

When these expectations, based on absence of position effects are compared with the results reported in tables 1, 2, 3 and 5, it is seen that the great majority of position effect values for the offspring of $ci/ci \times R(ci)$ crosses are positive, in contrast to an

expectation, based on the presence of triplo-4 flies, of negative values. All these positive differences are therefore truly expressions of position effects although the specific indexes sometimes may possibly be compounded of a typical position effect value and a superimposed triplo-4 value. Besides the positive means, however, some negative ones are also listed. These will be the subject of further discussion.

For the offspring of $+/+ \times R(ci)$ crosses the occurrence of triplo-4 individuals even in the absence of a position effect would yield positive values. Actually such values were found nearly exclusively, the few exceptions giving zero values or small negative values essentially not differing from zero. However, many of the positive values, including all those listed in table 1 are much larger than accountable by the presence of triplo-4 flies. In earlier work, (STERN 1943) with flies raised at 13°C the mean expressivity of $ci/ci/+$ males was found to be only .66 class intervals greater than that of $ci/+$. Most likely the difference would be considerably smaller with flies raised at 17°C, the temperature employed in the present experiments. It is concluded, therefore, that all values in table 1 are true expressions of position effects.

This conclusion is in line with the earlier one according to which the positive values for $R(ci)/ci$ phenotypes definitely indicate position effects, since it is reasonable to assume that an $R(ci)$ allele which gives a position effect with ci also shows the effect in combination with $+$. The same consideration also applies to many of the smaller positive values of $R(ci)/+$ phenotypes, particularly those in table 2. These positive values may likewise be regarded as due to position effects.

Direct proof for position effect underlying both negative $R(ci)/ci$ and the relatively small positive $R(ci)/+$ values was finally obtained in a series of special tests. Here, the $R(ci)/ci$ P males were simultaneously crossed to ci/ci or $+/+$ females and to $M-4/ey^D$ females. If the P male was triplo-4, the $M-4/ey^D$ females would produce non- ey^D non-Minute ($M-4/+/+$) offspring besides the expected ey^D and M offspring of diplo-4 males. Only cultures from proven diplo-4 males and ci/ci or $+/+$ females were scored and their position effect indexes entered in the tables as stated below. In addition, cytological preparations of some of the female offspring were made.

The following $R(ci)$ alleles were thus tested (P ♂♂ by progeny tests, F_1 ♀♀ cytologically):

Table 2, $R^{34}(ci)/+$ (second line). All progeny tested 12 P males were diplo-4, as well as all 15 of their cytologically studied $R(ci)/+$ offspring.

Table 2, $R^{42}(ci)/ci$ (second line). All 11 P males were diplo-4.

Table 2, $R^{37}(ci)/+$ (second line). All eight P males and 31 of their $R(ci)/+$ F_1 were diplo-4.

Table 3, $R^{16}(ci)/ci$ (second line). All nine P males and 18 of their $R(ci)/ci$ offspring selected from the phenotypic class 1 were diplo-4. (The selection of the nearly normal class 1 $R(ci)/ci$ flies from the—on the average—less normal progeny should have favored the discovery of possible triplo-4 individuals if these had been responsible for the negative index.)

Table 3, $R^{36}(ci)/ci$ (second and third lines). All 14 P males were diplo-4.

Table 3, $R^{45}(ci)/+$ (second line). All nine P males and 25 of their $R(ci)/+$ F_1 were diplo-4.

Table 3, $R^{45}(ci)/ci$ (third line). Three class 0 and seven class 1 F_1 were diplo-4.

Table 3, $R^{26}(ci)/+$ (second line). All nine P males and 31 of their $R(ci)/+$ F_1 were diplo-4.

Table 3, $R^{40}(ci)/+$. Three class 1 $R(ci)/+$ F_1 were (probably) diplo-4. (The selection of class 1 or 2 $R^{40}(ci)/+$ and $R^{39}(ci)/+$ flies from the otherwise nearly exclusively normal progeny should have favored the discovery of possible triplo-4 individuals if these had been responsible for the positive indexes.)

Table 5, $R^{39}(ci)/+$ (second line). Four of eight P males were diplo-4, the other four triplo-4. Among 27 class 1 and 2 $R(ci)/+$ F_1 of the diplo-4 parents were 26 diplo-4 and one triplo-4.

These progeny tests and cytological determinations establish for the cases studied, that negative values for $R(ci)/ci$ are not due to presence of triplo-4 flies, and that likewise even small positive values for $R(ci)/+$ are not due to presence of triplo-4 flies.

Tests for duplications

Some $R(ci)$ rearrangements produce viable individuals which in addition to two normal second and third chromosomes contain a duplication of the translocated tip of one of these chromosomes. Such flies are deficient for part of chromosome 4. Usually the duplication-carrying flies were not included in this report since they are phenotypically distinguishable from their sibs. However, this is not true for $R^{45}(ci)$ flies. Therefore special tests were made with this allele which had given the largest negative $R(ci)/ci$ indexes (table 3). In crosses of $ci/ci \times R^{45}(ci)/ci$, two phenotypically indistinguishable $R(ci)$ genotypes occur among the offspring, regular translocation heterozygotes and duplication-2, deficiency-4 ($Dp\ 2, Df\ 4$) flies. In addition normal ci/ci and lethal $Df\ 2, Dp\ 4$ zygotes are produced. Thus the theoretical ratio of $R(ci)$ to ci flies is 2:1. On the other hand, the expected ratio of these phenotypes in crosses of $ci/ci \times R^{45}(ci)\ Dp\ 2, Df\ 4$ is 1:1.

It was investigated whether the $Dp\ 2, Df\ 4$ individuals were more normal than regular $R(ci)/ci$ flies, since such a phenomenon could account for the observed negative expressivity values. F_1 males with $R(ci)$ phenotypes from $ci/ci \times$ regular $R^{45}(ci)/ci$ were classified according to degree of vein interruption and their progeny tested with ci/ci females. In their offspring either 2:1 or 1:1 ratios of $R(ci):ci$ were approximated. This permitted classification of the tested males into regular and $Dp\ 2, Df\ 4$ genotypes. The results (table 6) show that the majority of 44 fertile males were of regular genotype regardless of the degree of interruption. If the 13 sterile

TABLE 6
Tests for $Dp\ 2, Df\ 4$ in $F_1\ R(ci)$ males from $ci/ci \times R^{45}(ci)/ci$

Genotype of tested male	Phenotypic class		
	1	2	3
Regular.....	4	24 (+1?)	9 (+1?)
$Dp\ 2, Df\ 4$	0	1 (+2?)	2
Sterile.....	0	6	7

males consisted of regular and *Dp* 2, *Df* 4 genotypes in the same ratio as the fertile males, then no significant difference in vein interruption would be caused by presence of the duplication as compared to regular *R(ci)/ci*. Should, on the other hand, the sterile males be mostly of *Dp* 2, *Df* 4 genotype, then this genotype would tend to give more—and not less—extreme interruption than the regular genotype. In either case these tests establish that the negative index for *R⁴⁵(ci)/ci* is not due to the presence of *Dp* 2, *Df* 4 individuals. It is therefore the expression of a true position effect.

THE EFFECTS OF *R*(+) ALLELES HETEROZYGOUS WITH *CI*

Sixteen of the 17 *R*(+) alleles all of which were detected by means of their position effect followed the rules established by DUBININ, KHOVOSTOVA and their colleagues. In them the breakage points in chromosome 4 were located in the right arm, between the centromere and the locus of *ci*, and the breaks in chromosomes 2 or 3 in the euchromatic parts distal to the proximal fifth or fourth of the chromosome arm. The exception from the rule, *R²(+)*, had one of its breaks in the left arm of chromosome 4 and the other in region 82D of 3R, close to the centromere of chromosome 3. All *R*(+) translocations were simple reciprocals except *R³(+)*, *R¹⁴(+)* and *R¹⁵(+)*. For a description of *R³(+)* and *R¹⁵(+)* the reader is referred to STERN, MACKNIGHT and KODANI (1946). In *R¹⁴(+)* three breaks are involved: (1) in the middle of 60B of 2R, (2) just before 99B1 of 3R, and (3) in 101F. The resulting rearrangement is as follows: 2L, 2R to 60B; 99B1 to 100. 3L, 3R to i.b. 99B1; 101F to 102. 4L, base of 4R to 101F; 60B to F.

TABLE 7

The effects of 17 R(+) alleles. F₁ of ci ey^R ♀♀ × R(+) +/ci ey^R ♂♂

Number of <i>R</i> (<i>ci</i>)	Break in chromosomes		Adjusted expressivity <i>R</i> (+)/ <i>ci</i>		N
	4	2 or 3	♀♀	♂♂	
3*	101	2L a. 21D1	3.16†	3.32†	932
10	i. b. 102B1	2L b. 31F1	1.39	1.52	656
15*	101, 102C	2L 30A	3.08†	2.91†	552
11	i. b. 102B1	2R 47C	0.51	0.70	766
20	101	2R i. a. 55A	1.05	1.44	681
18	101F	2R i.b. 57F1	1.93	2.12	904
6	101	2R i. a. 59D	1.46	1.70	774
21	101F	2R i. a. 60E1	2.11	2.29	676
19	i. b. 102B1	3L b. 62D3	1.27	1.73	593
13	i. b. 102A	3L i. a. 65D1	2.33	2.43	770
2	left arm	3R 82D	3.03	3.26	1089
16	101F	3R a. 90E1	2.01	1.77	928
5	101F	3R i. a. 96E1	1.38	1.68	498
8	i. b. 102A	3R i. b. 96F6	1.42	1.90	673
9	101F	3R i. b. 97C1	2.43	2.57	492
12	101	3R i. b. 98F1	2.55	2.85	612
14*	101F	3R i. b. 99B1	1.54	1.70	685

* complex cytology

† see text

The mean values for the vein interruption of females and males of the different $R(+)/ci$ genotypes derived from crosses of $ci/ci \times R(+)/ci$ ranged from 1.03 to 3.44 and .95 to 3.46 respectively. These values are direct measures of the degree of position effects since typical heterozygotes, $+/ci$, raised at 26°C are normal, i. e. have a value of 0. The expressivity values for the $R(+)/ci$ flies are subject not only to intrinsic variability but also to secondary environmental or genetic modification. The existence of such factors is indicated by considerable correlation between the expressivity of the ci/ci female and male sibs of the different $R(+)/ci$ flies. The ci/ci values in 16 of the $R(+)$ series (exclusive of $R^{15}(+)$; see below) varied from 2.06 to 2.60 for females and from 2.34 to 2.97 for males. The over-all averages for 3786 ci/ci females and 3633 ci/ci males from these 16 series were 2.32 and 2.72. Since it is likely that the secondary modifiers of the expressivity of ci/ci also affect $R(+)/ci$ in a similar way, an approximate correction was made by adding to a given $R(+)/ci$ value (a), the difference between the average ci/ci value for all 16 experiments (b) and the ci/ci value of the given $R(+)$ experiment (c). The adjusted $R(+)/ci$ values, as entered in table 7, are thus equal to $a + (b - c)$.

The adjusted values still are not corrected for secondary variability due to occurrence of triplo-4 or viable duplication individuals. For two of the $R(+)$ alleles, $R^{15}(+)$ and $R^3(+)$, it is known that such individuals are frequent (STERN, MACKNIGHT and KODANI 1946). In the case of $R^{15}(+)$ the $R(+)$ phenotypes are diplo-4 $R^{15}(+)/ci$ but half of the ci sibs contain an $R^{15}(+)$ duplication which makes them triplogenic $R^{15}(+)/ci/ci$. Therefore, the expressivity value of the $R^{15}(+)/ci$ flies could not be adjusted to that of its ci sibs but was entered directly in table 7. In $R^3(+)$ cultures the $R(+)$ phenotype is represented by genotypes $R^3(+)/ci$ and $R^3(+)/ci/ci$. The triplogenic flies are more nearly normal than the diplogenic ones. The adjusted $R^3(+)/ci$ values in table 7—though the highest in the whole series—are thus underestimates of the actual position effect of $R^3(+)$.

With the exception of $R^2(+)$, the results with $R(+)$ alleles agree with those of earlier workers. Some specific comments will be made in the discussion.

Comparisons of the degrees of $R(ci)$ position effects in heterozygotes for ci and $+$

In a very general sense, a positive correlation exists between the degree of position effects for the different $R(ci)$ alleles in the combinations $R(ci)/ci$ and $R(ci)/+$. This is apparent from a survey of tables 1-3 and 5. The rearrangements with breaks to the left of the ci locus and in the distal regions of the long autosomes all give strong position effects in both types of heterozygotes (table 1). Most other rearrangements give weak effects, again in both types of heterozygotes (tables 2, 3, 5). Moreover, the series of rearrangements with breaks to the left of ci and in the chromocenter of the long autosomes (table 2, lower part) which as a group gives the relatively strongest position effects in $R(ci)/ci$ outside of those listed in table 1, also gives relatively strong effects in $R(ci)/+$.

In detail, however, the correlation in strength of position effects of $R(ci)$ alleles in heterozygotes with ci and $+$ breaks down in numerous instances. Thus, in table 1, $R^7(ci)$ in combination with ci shows a greater position effect than $R^{58}(ci)$, while in combinations with $+$ the reverse is true. If the numbers of the 14 $R(ci)$ alleles of

table 1 for which both types of heterozygotes have been studied are listed in order of increasing strength of position effect in $R(ci)/ci$ female heterozygotes one obtains the following seriation: $23 < 5 < 55 < 31 < 33 < 53 < 32, < 3 < 18 < 8 < 19 < 29 < 7 < 52$. Their order in $R(ci)/+$ female heterozygotes is found to be $7 < 5 < 19 < 55 < 3 < 52 < 53 < 33 < 8 < 32 < 23 < 31 < 29 < 18$, thus showing a striking lack of correlation for the two combinations. Similarly, the ranking of the 14 $R(ci)/ci$ male heterozygotes in order of effect is as follows: $23 < 55 < 53 < 3 < 33 < 31 < 18 < 32 < 19 < 29 < 7 < 52 < 8$. The non correlated sequence for $R(ci)/+$ male heterozygotes is $5 < 7 < 19 < 55 < 3 < 53 < 52 < 33 < 8 < 32 < 23 < 29 < 31 < 18$. Many other examples of independence between the degrees of effect in the two types of heterozygotes can be seen by comparing different $R(ci)$ alleles listed in different tables or within the same table of the set of tables 1-3 and 5.

It is true that the data are not strictly comparable since the different types of heterozygotes were obtained at various times so that the results may have been affected by divergent environmental or genetic modifying conditions. It is unlikely, however, that such secondary variation is sufficient to account for the many striking reversals in the order of position effects in the $R(ci)/ci$ and $R(ci)/+$ heterozygotes. This has been proven specifically for two $R(ci)$ alleles selected from those listed in table 1, namely $R^{52}(ci)$ and $R^{18}(ci)$. At 26°C the $R^{52}(ci)/ci$ flies are nearly exclusively of the most extreme phenotype (average expressivity for females 3.98-4.00, for males 3.99) while $R^{18}(ci)/ci$ are less extreme (average expressivity ♀ ♀ = 3.17-3.32; ♂ ♂ = 3.60-3.70). In consequence, as seen in table 1, and copied in line 1 of table 8, the degree of position effect for $R^{52}(ci)/ci$ is greater than for $R^{18}(ci)/ci$ (differences highly significant). In contrast to this, the degree of position effect of $R^{52}(ci)/+$ is strikingly smaller than of $R^{18}(ci)/+$, the latter showing the most extreme effect among all $R(ci)/+$ heterozygotes. In order to test the validity of these findings, the experiments were repeated by mating single $R^{52}(ci)/ci$ and $R^{18}(ci)/ci$ males simultaneously and separately to both ci/ci and $+/+$ females and raising the offspring of the different females in separate cultures. In this way secondary environmental and genetic variability was greatly reduced. The results are shown in line 2 of table 8. They are essentially the same as in the less controlled experiments: greater position effect of $R^{52}(ci)/ci$ than of $R^{18}(ci)/ci$ but smaller position effect of $R^{52}(ci)/+$ than of $R^{18}(ci)/+$. (In a third experiment (line 3, table 8) the same mating procedures were

TABLE 8

Comparisons of the degrees of position effects of $R^{52}(ci)$ and $R^{18}(ci)$. F_1 of $ci\ ey^R$ ♀ ♀ × $R(ci) +/ci\ ey^R$ ♂ ♂ (F_1 raised at 26°C except in April 1948 when 17°C was used) and F_1 of $+ ey^2$ ♀ ♀ × $R(ci) +/ci\ ey^R$ ♂ ♂ (F_1 raised at 17°C)

Date of Experiment	Index			Index			Index			Index		
	$R^{52}(ci) +/ci\ ey^R$		N	$R^{18}(ci) +/ci\ ey^R$		N	$R^{52}(ci) +/+ ey^2$		N	$R^{18}(ci) +/+ ey^2$		N
	♀ ♀	♂ ♂		♀ ♀	♂ ♂		♀ ♀	♂ ♂		♀ ♀	♂ ♂	
1944	1.70	1.28	1021	1.08	0.94	1394	1.44	1.63	827	2.49	2.62	613
Feb. 1948	1.78	1.38	497	1.02	1.21	230	0.91	0.92	139	2.83	2.50	40
April 1948	0.59	0.28	824	0.56	0.29	702	1.36	1.10	627	2.48	2.45	452

used as in the second, but both the $R(ci)/+$ and the $R(ci)/ci$ offspring were raised at 17°C. In this experiment again the $R^{52}(ci)/+$ values were smaller than the $R^{18}(ci)/+$ values. However, the $R(ci)/ci$ values have become nearly identical, due to the shifting at the low temperature of nearly all phenotypes into the most extreme class.)

THE EFFECTS OF $R(CI)$ HOMOZYGOTES

A number of different $R(ci)$ alleles were obtained as homozygotes, either in pure stocks or, if poorly viable or fertile, in stocks of the parentage $R(ci)/ey^D \times R(ci)/ey^D$ (ey^D usually lethal in homozygotes). In order to compare the expressivity of $R(ci)/R(ci)$ with that of ci/ci under similar conditions, cultures were simultaneously stocked with fertilized P females which would produce non- ey^D $R(ci)/R(ci)$ homozygotes (and sometimes $R(ci)/ey^D$ flies), and fertilized $ci\ ey^R/ci\ ey^R$ P females which would produce solely eyeless ci/ci homozygotes. No P males were placed into the culture bottles.

The mean expressivities of the $R(ci)/R(ci)$ and ci/ci homozygotes were determined and the differences entered in columns 2 and 3 of table 9. For purposes of comparison the differences between the mean expressivity of heterozygous $R(ci)/ci$ flies and their ci/ci sibs are listed in columns 4 and 5. As a control, cultures were prepared in which fertilized females from ci/ci and $ci\ ey^R/ci\ ey^R$ stocks were simultaneously permitted to produce offspring. The first row of table 9 shows that the $F_1\ ci/ci$ females were by .11 expressivity units more normal than the $F_1\ ci\ ey^R/ci\ ey^R$ females (negative difference!) while the $F_1\ ci/ci$ males were by .05 units less normal than the $F_1\ ci\ ey^R/ci\ ey^R$ males (positive difference). Both differences are small and, considering the general variability of expressivity, indicate that ci/ci and $ci\ ey^R/ci\ ey^R$ flies are equivalent in

TABLE 9

The effects of $R(ci)$ homozygotes. See text for methods used. The indexes of $R(ci)/ci$ heterozygotes have been copied from tables 1 and 2

Number of $R(ci)$	Index $R(ci)/R(ci)$		Index $R(ci)/ci$		N*
	♀♀	♂♂	♀♀	♂♂	
<i>ci</i> control	-.11	.05	.01	-.05	561, 180
52	.08	.04	1.70	1.28	555, 86
18	-.84	-.46	1.08	.94	211, 1779
18†	—	-.97	1.08	.94	69, 114
29	-.75	-1.24	1.34	1.04	218, 272
29†	—	-2.03	1.34	1.04	138, —
54	-.23	-.73	1.29	1.09	1672, 77
44	0	-.05	.54	.61	922, 596
28	.53	.36	.67	.63	150, 536
37	-.17	-.18	.45	.37	534, 722
48	-.04	-.04	.62	.50	271, 1984

* The first of the two numbers given under N represents the ci/ci or Rci/Rci , the second the $ci\ ey^R/ci\ ey^R$ individuals.

† See text.

expressivity. The differences between the expressivities of $ci +/ci ey^R$ and $ci ey^R/ci ey^R$ given in the last two entries of line 1, .01 and $-.05$ have been discussed earlier (p. 348).

Postponing briefly a consideration of the repeat experiments with $R^{18}(ci)$ and $R^{29}(ci)$ listed in rows 4 and 6, the table contains data on $R^{52}(ci)$, $R^{18}(ci)$, $R^{29}(ci)$ and $R^{54}(ci)$ which have their breaks to the left of ci and in a distal region of chromosome 3 (see table 1) and $R^{44}(ci)$, $R^{28}(ci)$, $R^{37}(ci)$ and $R^{48}(ci)$ in which one of the breaks is in the chromocenter of either chromosome 2 or 3 (see table 2). The first named four $R(ci)$ alleles produced a strikingly more extreme expressivity than ci/ci when acting in $R(ci)/ci$ heterozygotes, and the last four $R(ci)$ alleles in combination with ci also gave clearly more pronounced expressivity than ci/ci even though less extreme than the first group. Surprisingly enough not a single one of the $R(ci)/R(ci)$ homozygotes deviated as much from ci/ci as the corresponding $R(ci)/ci$ heterozygotes. On the contrary 11 of the 16 expressivity differences were not even intermediate in expressivity between ci/ci and $R(ci)/ci$ (positive indexes) but more normal than ci/ci (negative indexes).

The finding of a more nearly normal expressivity of $R(ci)$ homozygotes as compared to $R(ci)/ci$ heterozygotes and, in specific instances, as compared even to ci/ci homozygotes was confirmed by two experiments of somewhat different design included in rows 4 and 6 of table 9. The first of these deals with $R^{18}(ci)$. A series of single pair matings was made of non-eyeless flies from the $R^{18}(ci)$ stock which contained non-eyeless $R^{18}(ci)/R^{18}(ci)$ and $R^{18}(ci)/ci ey^R$ and eyeless $ci ey^R/ci ey^R$ flies. One hundred eighty-nine F_1 males were classified in regard to their expressivity and then progeny tested individually in matings to $ci ey^R/ci ey^R$ females. Six cultures were sterile. Among the fertile males, 69 gave only non-eyeless offspring and thus were $R(ci)/R(ci)$ homozygotes and 114 gave both non-eyeless and eyeless offspring and thus were $R(ci)/ci ey^R$. The distributions of the males over the array of expressivity classes is shown in table 10. It is seen that the $R^{18}(ci)/R^{18}(ci)$ phenotypes were greatly shifted toward normal as compared to the $R^{18}(ci)/ci$ phenotypes. The mean expressivities were 1.96 and 2.93 respectively, yielding a difference of $-.97$ as entered in table 9.

The second special experiment is concerned with $R^{29}(ci)$. This position allele was available in a homozygous $R^{29}(ci)/R^{29}(ci)$ stock. A classification of 138 males showed that 74 were without vein interruption (class 0), 43 in class 1, 21 in class 2 and none in the extreme classes, 3 or 4. Since this result is so strange it was felt desirable to verify the $R^{29}(ci)/R^{29}(ci)$ genotype of the males; all were mated to $ci ey^R/ci ey^R$

TABLE 10

Phenotypic distributions of $R^{18}(ci)/R^{18}(ci)$ and $R^{18}(ci)/ci$ males. Genotypes determined by progeny tests

	0	1	2	3	4
$R^{18}(ci)/R^{18}(ci)$	0	20	34	13	2
$R^{18}(ci)/ci ey^R$	0	1	23	73	17
Sterile	0	0	3	3	0

females. One third of the cultures were sterile but every fertile male produced only non-eyeless offspring. The progeny of four males, two belonging to class 0, and one each to classes 1 and 2 were classified in detail. They were all similar in giving extreme $R^{29}(ci)/ci\ ey^R$ phenotypes with a mean expressivity based on 800 flies of 3.59 for females and 3.63 for males, thus comparing well with the values of 3.52 and 3.48 obtained earlier and used in the determination of the position effect indexes for $R^{29}(ci)/ci$ listed in table 1. The mean expressivity of the 138 homozygous $R^{29}(ci)$ males was .62. Using the value of 2.65 for the mean expressivity of $ci\ ey^R/ci\ ey^R$ males as determined from 9927 male sibs of the $R(ci)/ci$ flies listed in tables 1-3 and 5 the shift of $R^{29}(ci)/R^{29}(ci)$ homozygotes toward normality is expressed in the difference -2.03 (table 9).

It is conceivable that some of the $R(ci)$ homozygotes were triplo-4 and contained a translocation-free $ci\ ey^R$ chromosome. This would account for a shift toward normality. However, this explanation cannot apply to at least the especially striking results with $R^{29}(ci)$. If the $R^{29}(ci)/R^{29}(ci)$ flies had been triplo-4 they would, in crosses to $ci\ ey^R$, be expected to have produced both diplo-4 and triplo-4 offspring with a resulting bimodality in venation phenotypes. No such bimodality was observed. Furthermore, in three crosses of $R^{29}(ci)$ homozygotes to $M-4/ey^D$, reported in the following section, the presence of a third chromosome 4 would have been discovered by the finding of non- $M-4$, non- ey^D offspring. No such flies occurred.

THE EFFECT OF $R(CI)$ IN HEMIZYGOTES

In order to study the expressivity of $R(ci)$ hemizygotes, females heterozygous for $ci\ ey^R$ and various $R(ci)$ alleles were mated to $M-4^5/+$ males. The $M-4^5$ progeny of

TABLE 11

Phenotypic distributions of $R(ci) +/M-4^5$ and $ci\ ey^R/M-4^5$ flies. The four subdivisions of this table correspond to the four types of $R(ci)$ alleles listed in tables 1, 2, 3, and 5

Number of $R(ci)$	$M-4^5 + w$										$M-4^5\ ey^R$			
	♀ ♀					♂ ♂					♀ ♀		♂ ♂	
	0	1	2	3	4	0	1	2	3	4	3	4	3	4
7	4	0	0	0	0	11	0	0	0	0	0	0	0	0
3	0	0	0	0	0	1	0	0	0	0	0	0	0	1
5	5	0	0	0	0	2	0	0	0	0	0	0	0	1
18	4	0	0	0	0	14	0	0	0	0	0	0	0	4
8	6	0	0	0	0	7	0	0	0	0	0	0	0	0
32	0	0	0	0	0	1	0	0	0	0	0	0	0	0
9	2	0	0	0	0	1	0	0	0	0	0	0	0	0
42	6	0	0	0	6	1	0	0	0	2	0	0	0	0
28	42	0	0	0	2	39	1	0	0	5	0	3	0	13
37	32	0	0	0	4	53	0	0	2	17	0	3	2	10
43	14	0	0	0	0	21	0	0	0	1	0	0	0	5
26	5	0	0	0	0	4	0	0	0	0	0	0	0	0
27	6	0	0	0	0	17	1	0	0	0	0	0	4	7
39	33	0	0	1	3	35	0	0	3	9	0	1	1	4

TABLE 12
Phenotypic distributions of $R(ci) +/M-4$ and $ci\ ey^R/M-4$ flies

Number of $R(ci)$	$R(ci)/M-4$										$ci\ ey^R/M-4$									
	♀ ♀					♂ ♂					♀ ♀					♂ ♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
44	0	0	0	0	0	2	0	1	2	2	0	0	0	0	0	0	0	0	0	0
48	0	0	1	11	2	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0
29	32	0	0	0	0	112	14	1	0	0	0	0	0	0	0	0	0	0	0	0
$ci\ ey^R$	0	0	0	0	0	0	0	0	0	0	2	9	62	303	49	1	1	36	310	108

such crosses should consist of two types, $R(ci)/M-4^s$ and $ci\ ey^R/M-4^s$. Since $M-4^s$ is a deficiency for both ci and ey , the first type is expected to have large non-eyeless, the second the smaller "eyeless" eyes. It was found that eyeless in hemizygous state often is poorly expressed, the eyes being relatively large. This fact introduces uncertainties of classification. The viability of both types is very low.

The successful crosses, in which $R(ci)/M-4^s$ and, usually, also $ci\ ey^R/M-4^s$ offspring were obtained, include $R(ci)$ examples of all four main groups of translocation alleles (see tables 1, 2, 3 and 5). The results are somewhat ambiguous (table 11). While all $ci\ ey^R/M-4^s$ flies belong—as expected from earlier work with hemizygous ci —to the most extreme classes 3 and 4, the non-eyeless $M-4^s$ flies fell into two phenotypic groups. In some $R(ci)$ combinations all round-eyed $M-4^s$ flies were completely normal in venation (class 0) or belonged to the nearly normal class 1. In others, the majority of the flies was normal while a minority belonged to the extreme classes 3 and 4. In the absence of a better explanation it may be assumed that the non-eyeless flies with extremely interrupted veins are normal eyed overlaps of the $ci\ ey^R/M-4^s$ genotype and therefore actually belong to this genotype. If this interpretation is correct, then all $R(ci)/M-4^s$ genotypes listed would give exclusively normal or nearly normal venation. This is very unexpected if one recalls the extreme expressivity of both hemizygous ci and often of $R(ci)/ci$ heterozygotes. However, it falls in line with the shift toward normality of several of the $R(ci)$ homozygotes.

Additional data on $R(ci)$ hemizygotes were obtained from crosses of three $R(ci)$ homozygotes to $M-4/+$ or $M-4/ey^D$ males (table 12). Altogether only seven $R^{44}(ci)/M-4$ males were obtained, distributed over classes 0, 2, 3 and 4. Similarly 21 $R^{48}(ci)/M-4$ flies were of the strongly interrupted classes 2, 3 and 4. Thus, they behaved similarly to the $ci/M-4$ flies as obtained in a special control experiment (table 12 last line). On the other hand, $R^{29}(ci)/M-4$ flies were mostly normal. Their expressivity values, 0 for females and .13 for males were considerably smaller than the values for $R^{29}(ci)/R^{29}(ci)$ homozygotes from a simultaneous experiment reported in table 9 which were .52 for females and 1.27 for males (underlying the indexes $-.75$ and -1.24 as reported there).

THE EFFECTS OF $R^x(CI)/R^y(CI)$ COMPOUNDS

Flies heterozygous for two different $R(ci)$ alleles were obtained by mating homozygous $R^x(ci)/R^x(ci)$ to $R^y(ci)/R^y(ci)$ flies or, in a few cases to $R^y(ci)/ey^D$, where $R^x(ci)$

and $R^y(ci)$ signify two different $R(ci)$ alleles. In order to compare the expressivity of the $R^x(ci)/R^y(ci)$ compounds with that of ci/ci a similar method was used as in the experiments with $R(ci)$ homozygotes. It consisted of placing into the culture bottles fertilized females which would produce $R^x(ci)/R^y(ci)$ offspring, as well as $ci\ ey^R/ci\ ey^R$ females fertilized by males of the same genotype.

Three groups of $R^x(ci)/R^y(ci)$ compounds were studied, namely compounds (1) between $R(ci)$ alleles both of which had their breaks distally in a long autosome, (2) between one $R(ci)$ allele with a break distally and another $R(ci)$ allele with the break in the chromocenter of a long autosome, and (3) between $R(ci)$ alleles both of which had their breaks in the chromocenter of a long autosome (table 13). All $R(ci)$ alleles used had the break in chromosome 4 proximal to the ci locus.

Without exception the 42 female and male compounds were closer to normal than one or both of the constituent $R(ci)$ alleles when heterozygous with ci . Thus, in group (1) of compounds, involving $R^{52}(ci)$, $R^{18}(ci)$ and $R^{54}(ci)$, the expressivity differences between $R(ci)/ci$ and ci/ci varied between .94 and 1.70 (table 1) while those

TABLE 13
The effects of $R^x(ci)/R^y(ci)$ compounds. See text for methods used

Group	Numbers of $R(ci)$'s	Breaks in regions†	Index $R^x(ci)/R^y(ci)$		N*
			♀♀	♂♂	
(1)	52/18	76, 86	.19	.17	1349, 761
	52/54	76, 87	.39	.39	694, 951
	54/18	87, 86	.31	.38	1405, 752
(2)	52/44	76, chrc. 2	.67	.60	361, 963
	52/28	76, chrc. 3	.12	.18	971, 521
	52/37	76, chrc. 3	.26	.14	224, 981
	52/48	76, chrc. 3	— .11	— .10	142, 555
	18/44	86, chrc. 2	.61	.42	645, 792
	18/28	86, chrc. 3	.31	.34	620, 1074
	18/37	86, chrc. 3	.22	.45	468, 956
	18/48	86, chrc. 3	.10	.22	857, 818
	54/44	87, chrc. 2	.41	.33	759, 547
	54/28	87, chrc. 3	.31	.21	874, 541
	54/37	87, chrc. 3	.17	.32	805, 639
	54/48	87, chrc. 3	.45	.54	359, 537
(3)	44/28	chrc. 2, chrc. 3	.07	— .03	361, 630
	44/37	chrc. 2, chrc. 3	— .07	.05	631, 760
	44/48	chrc. 2, chrc. 3	.60	.40	336, 494
	28/37	chrc. 3, chrc. 3	— .07	— .36	664, 443
	28/48	chrc. 3, chrc. 3	— .07	.24	465, 315
	37/48	chrc. 3, chrc. 3	— .46	— .16	648, 433

* The first of the two numbers given under N represents the $R^x(ci)/R^y(ci)$, the second the $ci\ ey^R/ci\ ey^R$ individuals.

† The breaks listed refer to the long autosomes. All breaks in chromosome 4 were proximal to ci . For details consult tables 1 and 2.

between $R^x(ci)/R^y(ci)$ varied between .17 and .39 (table 13). In group (2) of compounds involving the $R(ci)$ alleles of the first group in combination with $R^{44}(ci)$, $R^{28}(ci)$, $R^{37}(ci)$ and $R^{48}(ci)$, the expressivity differences of the compounds ranged from $-.11$ to $+.67$ which is again below the minimum difference involving the first-named $R(ci)$ alleles. Moreover, the majority of the $R^x(ci)/R^y(ci)$ compounds in group 2 were also more normal than the $R^{44}(ci)$, $R^{28}(ci)$, etc. alleles in heterozygotes with ci . The differences between $R^{44}(ci)/ci$, etc. and ci/ci varied between .37 and .67 (table 2), while among the 24 differences between $R^x(ci)/R^y(ci)$ of the group (2) and ci/ci , 16 were below .37 (table 13). Finally, in group (3) of $R^x(ci)/R^y(ci)$ compounds 10 out of the 12 differences ranged between $-.46$ and $+.24$ and thus were smaller than the differences between ci/ci and any of the $R(ci)$ alleles in this group when heterozygous for ci . Seven of these 10 values were negative which corresponds to a phenotype more normal than that of ci/ci .

The shift toward low expressivity of $R^x(ci)/R^y(ci)$ compounds as compared to $R(ci)/ci$ heterozygotes containing the constituent $R^x(ci)$ or $R^y(ci)$ is alike in direction to that of $R(ci)/R(ci)$ homozygotes. A comparison of the data in tables 9 and 13 shows that the shift toward normality for compounds, group (1), involving $R^{82}(ci)$, $R^{18}(ci)$ and $R^{54}(ci)$ is less striking than for the homozygotes of these alleles and that the same holds true for most, but not all, compounds of groups (2) and (3).

Differences of mean expressivity in females and males

The mean expressivity for ci/ci is lower in the female than in the male. In two independent series of experiments at 26°C, the difference between the values for the two sexes was found to be .32, and .42 respectively (table 14, b, f. See footnote to

TABLE 14
Differences of mean expressivity in females and males of different genotypes

	Numbers of diff. (♀ — ♂)			Mean diff. (♀ — ♂)*			
				positive		negative	
	pos	neg	0	mean	range	mean	range
(a) Means $R(+)/ci$	3	14	0	.20	.08-.36	.16	.01-.30
(b) Means $ci\ ey^R/ci\ ey^R$	0	17	0	.23	.13-.28	.32	.15-.49
(c) Indiv. cultures of (a) excl. those $R(+)$ with positive mean	12	58	3	—	—	—	—
(d) Indiv. cultures of (b)	4	82	0	—	—	—	—
(e) Means $R(ci)/ci\ ey^R$	2	41	1	.04	.03-.04	.33	.06-.63
(f) Means $ci\ ey^R/ci\ ey^R$ (sibs of e)	0	44	0	.42	.37-.47	.42	.19-.70
(g) Indiv. cultures of (e) excl. those $R(ci)$ with positive mean	12	242	1	—	—	—	—
(h) Indiv. cultures of (f)	8	265	0	—	—	—	—
(i) Means $R(ci)/+$ 17°C	6	32	4	.04	.01-.11	.11	.01-.34
(j) Means $ci/+$ 17°C (sibs of (i))	0	26	13	.02	0-.06	.03	.01-.09

* The mean differences (♀ — ♂) are given separately for cultures where the mean differences for $R(+)$ or $R(ci)$ sibs were positive and for those where they were negative. The combined mean difference (♀ — ♂) for ci/ci of line (b) was .32.

table.) In 86 individual cultures of the first series, only four and in 273 cultures of the second series, only eight had a higher value for females than for males (d, h).

$R(ci)/ci$ flies show a similar differential with only two out of 44 differences of mean values and only 12 out of 255 cultures in favor of the females (e, g). The two positive differences out of 44 were very small, and the single case with equal expressivity for the two sexes was $R^{52}(ci)/ci$ in which nearly all flies belonged to the most extreme class 4, the mean expressivity for females and males being 3.99.

The situation is different for the $R(+)/ci$ phenotypes (a, c). Among the 17 different genotypes three showed a higher expressivity for the females, and the same was true for 12 among 73 cultures. Moreover, the mean positive difference, in favor of the females, was relatively large, namely .20, and the mean negative relatively small, namely .16. It thus appears that the expressivity in the two sexes of the $R(+)/ci$ types is shifted toward a positive value, as compared to ci/ci and $R(ci)/ci$. This shift seems not to be related to the mean degree of expressivity since most values for the three genotypes lie within the same range and since the $R(+)/ci$ genotypes with positive and negative values for the difference in expressivity for the two sexes are randomly distributed over the range of mean expressivities.

There is a suggestion of a similar shift for the $R(ci)/+$ values (i, j). A control series of 39 $ci/+$ values contained no positive value (among the 13 values with no difference between the sexes there were 11 with expressivity 0, i.e. only normal flies, and two values with mean expressivities .004 and .01). In contrast, the 42 $R(ci)/+$ values contained six positive ones.

The reason for the relatively increased expressivity primarily of the $R(+)/ci$ combinations in the female sex is not clear. It seemed possible that the shift was due to secondary causes, such as greater frequency of triplo-4 flies in one than in the other sex. If, for instance, the P males frequently had been $R(+)/ci/ci$, and if preferential segregation had occurred such as to make (a) $R(+)$ alone segregate more frequently with the X than with the Y chromosome and/or (b) $R(+)/ci$ segregate less frequently with the X than the Y chromosome, then the $R(+)/ci$ progeny would contain a surplus of females and/or the $R(+)/ci/ci$ progeny a surplus of males. Since $R(+)/ci$ is phenotypically more extreme than $R(+)/ci/ci$ the difference in expressivity in the two sexes would be diminished or even reversed. However, this hypothetical mechanism of preferential segregation in supposedly triplo-4 P males would also change the difference in expressivity of the ci sibs. These would consist of ci/ci and $ci/ci/ci$ flies. This consequence alone would result in a more normal mean expressivity for which, with one exception, there is no evidence. Moreover, the preferential segregations would result in a surplus of the complementary classes of $ci/ci/ci$ sons over $ci/ci/ci$ daughters, and of ci/ci daughters over ci/ci sons which would decrease the over-all difference in ci expressivity for the two sexes. This expectation is not borne out by the facts (table 14, b). The mean of the 14 values from ci sibs of $R(+)/ci$ combinations with negative values does not differ significantly from the mean of the three values from ci sibs of $R(+)/ci$ combinations with positive values.

If no secondary mechanism of the kind outlined can be found to explain the reduction or reversal of sexual difference in the expressivity of $R(+)$ alleles in com-

bination with *ci* as contrasted with *ci* itself and, probably, with *R(ci)* alleles then the cause must lie in an intrinsically different reaction of *R(+)* alleles in development. No attempt will be made to speculate on the nature of such a different reaction.

DISCUSSION

LEWIS' (1950) penetrating review of the phenomenon of position effect contains a description and analysis of many facts and of the interpretations offered by various authors. In cases of the stable "S-type" position effects the concept of localized interactions between genes and gene products was elaborated into a fruitful theory. The variegated "V-type" position effects to which, with some uncertainty, the *ci* effect may be assigned, have not yet been amenable to a successful unifying treatment. While the present report adds new facts which permit the formulation of some rules no deeper insight into the mechanism of position effect at the *ci* locus can be presented.

The data on *R(ci)/ci* and *R(ci)/+* heterozygotes show that position effects at the *ci* locus occur not only with rearrangements involving a break between the centromere and the locus of *ci* but that breaks distal to the locus also result in position effects. Moreover, contrary to statements by earlier workers based on the results with selected *R(+)* alleles proximal as well as distal regions of chromosomes 2 and 3 change the effect of *ci*, though to different degrees, when brought in association with this locus. This had been foreseen by SIDOROV (1941) who expressed the opinion that "any break near the *ci* gene practically causes some change in its action". The validity of this statement has now been established by the study of an unselected series of *R(ci)* alleles.

No systematic search for heretofore undetected, presumably weak position effects involving *R(+)* has been made. In analogy with the findings on *R(ci)* it may be assumed that *R(+)* position effects are likewise produced not only by translocation of the *ci*⁺ allele to distal breaks in chromosomes 2 and 3 but also by other types of rearrangements between chromosome 4 and with 2 or 3. There are a few data which support this assumption. Thus, SIDOROV (1941) studied two *R(+)* alleles with proximal breaks in chromosome 3 (and, presumably, proximal breaks in chromosome 4) which in the combination *R(+)/ci* did not produce position effects. The same *R(+)* alleles, however in the combinations *R(+)/R(ci)* produced noticeable expressivity shifts. Further indication of the production of *R(+)* position effects by rearrangements with chromosomes 2 or 3 other than translocation of *ci*⁺ to distal regions is provided by the *R*²(+) allele. Here, the break in chromosome 4 was in the left arm so that the continuity of the *ci* locus with its neighboring material in the right arm was not disturbed. Nevertheless, the translocation of a distal section of chromosome 3 to the left arm, i.e. beyond the centromere, resulted in a position effect. An alternative interpretation of *R*²(+) is that a mutation of *ci*⁺ to a *ci* allele was induced simultaneously with the rearrangement (LEWIS 1950).

Among the *R(ci)* alleles in which *ci* has become translocated to chromosome 2 or 3 (tables 1, 2) three different groups can be distinguished jointly by (a) their degrees of position effect in *R(ci)/ci* and *R(ci)/+*, and (b) the location of the break in chromosome 2 or 3. Group 1 gives usually striking effects, group 2 weak effects or ab-

sence of effect and group 3 intermediary effects. All these position effect indexes, with two probably insignificant exceptions, were positive. In group 1 the breaks are distal to the first proximal fifth or quarter of the chromosome arm involved. In group 2 the breaks are close to though still distal to the chromocentral region. In group 3 the breaks are located within the chromocenter. The single partial exception is $R^9(ci)$ listed in table 2, among group 2. While its $R^9(ci)/ci$ index is low as expected in group 2 its $R^9(ci)/+$ index is of intermediary strength. In reality, as pointed out earlier, for $R^9(ci)$ the break in chromosome 2 is more distal than all other relatively proximal breaks of the other alleles in group 2. $R^9(ci)$ thus forms a connecting link between groups 1 and 2 both as to degree of effect and location of break. The relation between degree of position effect and location of break in the long autosomes is illustrated for $R(ci)/ci$ females in figure 3 in which the abscissa represents chromosomes 2 and 3—superimposed upon one another—from the left ends through the chromocenter to the right ends, and the ordinate the degree of position effect as measured

FIGURE 3. Position effects of $R(ci)/ci$ heterozygous females. Breaks in chromosome 4 between centromere and ci . Relation between location of the break in chromosome 2 or 3 and degree of position effect. The abscissa represents superimposed upon one another regions 21-60 of chromosome 2 and 61-100 of chromosome 3. The numbers designate the $R(ci)$ alleles. Solid circles = $R(ci)$ alleles involving chromosome 2. Open circles = $R(ci)$ alleles involving chromosome 3.

by the index. The same kind of diagrams with very similar results can be constructed from the data of tables 1 and 2 for $R(ci)/ci$ males, and for $R(ci)/+$ females, and males.

The locus of ci , in its normal position in chromosome 4, is distal to the centromeric region but still close to it. Indeed, the whole short chromosome 4 may be regarded as equivalent in reference to its neighborhood to the centromere to the proximal regions of chromosomes 2 and 3. It is noteworthy that the $R(ci)$ alleles of group 2 which give the weakest position effects are the ones in which the ci locus has been translocated to a proximal region of chromosomes 2 or 3. Apparently, the new location of the translocated ci allele is physiologically similar enough to its normal location so as to result in no change or only a slight change in effect. In contrast to translocation into a proximal region, the $R(ci)$ alleles of groups 1 and 3 which are transferred to either a distal or a chromocentric region show greater changes in their effects.

These facts may be interpreted in the sense that the normal locus of ci lies in a region which is similar to the heterochromatic sections adjacent to the centromeres of chromosomes 2 and 3. Striking position effects of the ci alleles thus result from their juxtaposition to euchromatic sections. In this respect the ci position effects resemble those of light (l) in chromosome 2 (SCHULTZ 1936). Variegation caused by position effect at this locus is suppressed in the X0 male and enhanced in individuals with extra Y chromosomes. In regard to ci position effects, the role of the Y chromosome is not clear. KHVOSTOVA (1939) has stated that $R(+)$ position effects "become(s) mostly more pronounced" in XXY females, but the author herself concludes that "the data are insufficient". On the other hand, ALTORFER (1951) has shown that some $R(ci)$ position effects are enhanced in X0 males while others remain unchanged. The enhancement of ci effects contrasts with the suppression of light effects and thus is similar to the enhancement in regard to position alleles of euchromatic loci brought into heterochromatic neighborhoods.

Within each of the three groups of position alleles represented in figure 3 there is great variation of degree of position effects from one $R(ci)$ allele to another, particularly in group 1. This variability may be due partially to secondary causes but much of it presumably depends upon the specific location of the various breakage points. No clear correlation between degree of effect of $R(ci)$ alleles and location of presumed intercalary heterochromatic regions is apparent though HANNAH (1951) pointed out a relation between breaks associated with $R(+)$ position effects and intercalary heterochromatin.

Had a correlation been found within each group between specific location of break and degree of position effect it could have related to part of the data only. This is so because there is largely independence between the degree of effect of the $R(ci)$ alleles in heterozygotes with ci and the degree of the same $R(ci)$ alleles in heterozygotes with $+$. This lack of correlation in effects points to qualitative differences in the developmental reactions of the different $R(ci)$ alleles or their products. A suggestion of qualitative differences in the nature of $R(+)$ alleles as compared with $+$, ci and $R(ci)$ is also seen in the reduction in $R(+)/ci$ of the difference of expressivity in the two sexes or even a reversal in direction of the difference.

Different $R(+)/ci$ heterozygotes are equally variable in their position effects as

heterozygotes involving different $R(ci)$ alleles. This variability has been discussed previously by DUBININ and SIDOROV (1934 a, b) and KHVOSTOVA (1939) and is borne out by the additional data of table 7 of the present paper. Similar to the $R^9(ci)$ allele which is associated with a break at 38B1 in chromosome 2 and which gave a smaller position effect index than the $R(ci)$ alleles with more distal breaks, DUBININ and SIDOROV's $R(+)$ -398, KHVOSTOVA's $R(+)$ -III-IV/1, and our $R^{11}(+)$ allele have all relatively proximally located breaks in chromosomes 2 or 3 and, simultaneously, relatively weak position effects. For the more distally located $R(+)$ alleles our data agree with KHVOSTOVA's who concluded that "a definite connection between the degree of expression of ci and the location of the breaks could not be established" (translated).

One of the unexpected findings concerns $R(ci)$ alleles in which the break in chromosome 4 had occurred distal to the ci locus so that the tip of chromosome 4 became replaced by a section of chromosome 2 or 3 (tables 3, 5). It is not surprising in itself that position effects result from such translocations since position effects due to breakage distal to a locus had earlier been shown to be produced in *D. melanogaster* for curled (PANSIN 1935), and position effects due to breakages on either side of a locus had been described for Notch (see HANNAH 1951) and, in *D. virilis*, for peach (BAKER 1953). It is not surprising either that the effects are usually weak since it could be assumed that the undisturbed relation between the chromocentral region of chromosome 4 and the ci locus would permit a relatively unchanged effect. This expectation was indeed fulfilled for 11 out of the 17 $R(ci)$ alleles with distal breaks in chromosome 4. In ten out of 11 cases a section or most of the right arm of chromosome 3 had been translocated to chromosome 4 and in the eleventh case most of the right arm of chromosome 2 beginning with the proximal region 44C. The unexpected finding refers to the six $R(ci)$ alleles in which a distal section of the right arm of chromosome 2, from as relatively proximal as 46 to as distal as 58D had been jointed to chromosome 4. In all of these six cases the $R(ci)/ci$ phenotypes were shifted toward normal as compared to ci/ci , resulting in a negative position effect index. It thus seems as if the distal parts of the right arm of chromosome 2 are, in some essentially unknown respect, different from all tested sections of either arm of chromosome 3. (No comparison with the left arm of chromosome 2 is available).

Negative indexes are not restricted to the last named group of $R(ci)$ alleles. They are found among $R(ci)/R(ci)$ homozygotes, $R(ci)$ hemizygotes, and $R^x(ci)/R^y(ci)$ compounds. Furthermore, the phenotypes of the tested homozygotes were all shifted toward normal as compared with $R(ci)/ci$, the phenotypes of some of the hemizygotes were completely normal, and those of all compounds were closer to normal than one or both of the constituent $R(ci)$ alleles when heterozygous with ci . Since the mutant ci allele is less effective than normal ci^+ alleles in leading to uninterrupted venation, the negative indexes signify a partial or complete restoration, in certain genotypes, of effectiveness of the ci allele in translocations. It may be mentioned that somewhat similar paradoxical situations concerning degrees of dominance have been discovered in the mouse (GRÜNEBERG 1953; older literature is cited in this publication). The gene *mi* for microphthalmia, in homozygotes, is much more strongly expressed than the allele Mi^{wh} in homozygotes. However, in the heterozygotes with the normal

allele *mi* has a much weaker effect than *Mi^{wh}*. Likewise, the expression of the gene *W* for macrocytic anemia is much more extreme than of the allele *W^v* in the homozygotes, but *W/+* heterozygotes have a normal blood picture while *W^v/+* mice are macrocytic.

The present data on homozygotes, hemizygotes and compounds include solely *R(ci)* alleles in which *ci* has been translocated to chromocentral or distal regions of chromosomes 2 or 3. It will be necessary in the future to fill various gaps in information particularly regarding the phenotypes of homozygotes for those *R(ci)* alleles in which the right arm of chromosome 2 has been translocated to chromosome 4, broken to the right of *ci*. It should also be kept in mind that possible explanations for negative indexes may be found which may reduce the apparent shifts toward normality to unimportant phenomena due to at present unknown secondary causes.

SUMMARY

1. The degrees of vein interruption are reported for 43 unselected *R(ci)* alleles in heterozygotes with *ci* and +, and for 17 *R(+)* alleles selected for showing position effects in heterozygotes with *ci*. Certain *R(ci)* homozygotes and hemizygotes, and *R^x(ci)/R^y(ci)* compounds have also been studied. Position effect indexes are calculated. A positive index signifies greater phenotypic deviation from normality than that of the corresponding genotype without a position allele. A negative index signifies lesser deviation from normality.

2. On the basis of several deficiencies and duplications, and of a difference in degree of position effect as related to location of a break in chromosome 4 the salivary chromosome band 102B1 or its immediate surroundings are considered as being the *ci* locus.

3. *R(ci)/ci* and *R(ci)/+* heterozygotes from translocations of *ci* (a) to distal regions of chromosomes 2 or 3 gave striking positive position effects; (b) to proximal regions, slightly distal from the chromocenter, gave no or weak positive position effects; (c) into the chromocenter gave positive position effects of intermediate degree.

4. *R(ci)/ci* and *R(ci)/+* heterozygotes involving translocations of distal or proximal plus distal parts of chromosomes 2 or 3 to chromosome 4, with a break to the right of *ci*, gave from no to intermediary position effects. In *R(ci)/ci* heterozygotes all six *R(ci)* alleles in which distal regions of the right arm of chromosome 2 were translocated to the *ci*-containing part of chromosome 4 gave negative position effects.

5. Genetic and cytologic tests of several *R(ci)* genotypes rule out triplo-4 individuals as the cause of the negative or of weak or intermediary positive position effect indexes.

6. The group of *R(ci)* alleles with strong or intermediary positive position effects in *R(ci)/ci* heterozygotes also gave strong or intermediary positive effects in *R(ci)/+*. However, within these as well as the other groups no correlation was apparent between strength of effect in *R(ci)/ci* and *R(ci)/+*.

7. In conformity with the results of earlier workers on *R(+)* alleles selected for showing position effects 16 out of the 17 *R(+)* alleles represented translocations of *ci*⁺ to distal regions of chromosomes 2 or 3. The seventeenth *R(+)* allele had one of

its breaks in the left arm of chromosome 4 and a translocation of most of the right arm of chromosome 3 to chromosome 4.

8. $R(ci)$ homozygotes as compared to the corresponding $R(ci)$ heterozygotes were shifted toward normal. Some were even shifted toward normal beyond ci homozygotes (negative indexes!).

9. Some $R(ci)$ hemizygotes were similar in phenotype to ci hemizygotes; others were completely normal.

10. $R^x(ci)/R^y(ci)$ compounds were closer to normal than one or both of the constituent $R(ci)$ alleles when heterozygous with ci . Some compounds gave negative indexes relative to ci/ci .

11. In females the expressivity of ci and $R(ci)$ in various combinations is less than in males. $R(+)/ci$ genotypes tend to show a lessened difference in the degree of expressivity of the two sexes or even a reversal in direction.

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