[36] In Morgan, T. H., Bridges, C. B., and Schultz, J., *Yearbook Carnegie Inst.*, No. 36, 298–305 (1937).

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[38] It is possible, however, that this deviation is somewhat misleading, for the change in phenotype may be broken down into two components—a change from wild type to brown and a change from homogeneous eye color to variegated. The former change is more marked in homozygotes than in heterozygotes, but the latter is diminished.

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[40] Caspersson, T., *Naturwiss.*, **31/32**, 514–515 (1940).

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[43] This paper was read prior to publication by Drs. M. Delbrück, Th. Dobzhansky, H. B. Glass, B. McClintock, H. J. Muller, C. Stern and several members of this department to whom we wish to express our thanks.

MATERIALS FOR THE STUDY OF THE POSITION EFFECT OF NORMAL AND MUTANT GENES

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The majority of recent studies on the position effect of genes in *Drosophila* have been concerned with normal alleles of various loci. Different kinds of effects seem to have resulted from change in position. In many cases the phenotypic expression of a normal gene has not been altered by a change of position. Some cases, however, have been observed in which the normal allele assumed a dominant mutant action (e.g., the dominant eye color, Plum). Other observations have indicated that the dominance of a normal allele may be diminished (e.g., cubitus interruptus), or that a normal allele may become more or less inactivated (e.g., white and Notch series). Few generalizations could be made from these results. It was thought, therefore, that some insight might be gained from a study which would attempt to correlate data derived from investigations of change in position of both a normal gene and its mutant alleles. The following represents a first report on such studies.[1]

Material.—The locus chosen was that of cubitus interruptus (*ci*) in the fourth chromosome of *D. melanogaster*. In 1934 Dubinin and Siderov[2] showed that many translocations involving the fourth chromosome cause an unusual change in the effect of the normal allele originally present. While this allele in its new position, both in hemi- or homozygous constitution, still produces a normal phenotype, it causes in heterozygous combina-

tion with the recessive mutant allele, *ci*, the appearance of the mutant vein character *ci*, an interruption of the cubital vein.

In order to obtain an unselected sample of translocations involving the fourth chromosome and the mutant allele *ci*, males homozygous for *ci* were x-rayed at 4000 r,[3] and crossed to females containing dominant marker genes in their second and third chromosomes. More than 20,000 F_1 males were individually back-crossed to *ci* $ey^R/ci\ ey^R$ females in order to test for the presence of translocations.[4] Fifty-five translocations involving the fourth chromosome and one or more of the chromosomes *Y*, II or III were found, and stocks of all of them established. Heterozygotes were obtained between all translocations and the allele *ci* in its normal position. About half of the translocations proved to give a more extreme phenotype in combination with *ci* than the typical mutant homozygote *ci/ci*. Five translocations of this group form the basis of the experiments to be reported. These five translocations were selected among those which, during an early stage of these studies, were found to exhibit the position effect described in the following section.

We shall use the symbol *R(ci)* for any rearrangement (*R*) which leads to a position effect of the mutant allele *ci*. Correspondingly *R*(+) will be used to denote a rearrangement giving a position effect of a normal allele +. *R(ci)* and *R*(+) may be referred to as "position alleles" since they behave as alleles of genes like *ci* and + in the typical position.

Heterozygotes of Position Alleles R(ci) and a Normal Allele.[5]—It was discovered that at room temperature some of the heterozygous $R(ci)/+^C$ individuals showed slight degrees of the *ci* phenotype, in contrast to $ci/+^C$ heterozygotes which at this temperature range are all normal.[6] In order to obtain reliable data, heterozygotes were raised at a lower temperature, 18–19°, which is known to intensify the expression of the *ci* phenotype. Table 1 shows that all five position alleles *R(ci)*, in combination with $+^C$, gave a larger number of individuals with the mutant phenotype than did $ci/+^C$ flies. Furthermore, while no individual of the latter consitution deviated more from normality than Class 0, which still shows uninterrupted though thinned veins, many flies of the constitution $R(ci)/+^C$ belonged to the more extreme Classes 1, 2 and 3.

The five translocations differ in the intensity with which they cause the mutant phenotype. For the present, two main groups of *R(ci)* alleles will be distinguished: (1) *R(ci)* –8 and –48 in which the phenotypic distribution of the heterozygotes $R(ci)/+^C$ deviates rather slightly from that of $ci/+^C$, Class *N* still representing 80 per cent or more of all individuals; and (2) *R(ci)* –23, –29 and –32 in which the deviations from normality are larger with only 60 per cent or less of the individuals belonging to class *N*.

Data from a preliminary cytological analysis of these translocations,

undertaken by Dr. H. D. Stalker, are included in table 1. Of the two translocations with weak expression one occurred near the chromocenter of chromosome 3 and one near the middle of 3*L*, while all three translocations with strong expression were located within close range of each other, about one-third the distance from the distal end of 3*R*. All breaks in chromosome 4 presumably lie proximal to 101*F*, i.e., between the centromere and the locus of *ci*, as described for the *R*(+) position alleles by Khvostova and Gavrilova (1935).[7]

Heterozygotes of Position Alleles R(ci) and ci.—As stated above, the five *R*(*ci*) translocations were chosen from those which in the combination *R*(*ci*)/*ci* produce a more extreme *ci* phenotype than the non-translocated *ci*/*ci* homozygote. Specific data permitting a comparison between *R*(*ci*)/*ci* and *ci*/*ci* sibs will be reported elsewhere, but in summary it may

TABLE 1

F_1 NON-ey^D OF WILD TYPE "CANTON" $(+^C/+^C) \times ci\ ey^R/ci\ ey^R$ OR $R(ci)/ey^D$* 18°C.

HETEROZYGOUS FOR $+^C$ AND:	BREAK IN CHROMOSOME 3 NEAR SALIVARY CHROMOSOME REGION:	PHENOTYPIC CLASSES† ♀♀ N	0	1	2	3	♂♂ N	0	1	2	3
ci ey	...	484	1	..	.	..	458	18	..	.	..
R(*ci*) – 8‡	69/70	129	16	5	.	..	157	16	5	.	..
R(*ci*) – 48	Chromocenter	174	10	..	.	..	153	17	..	.	..
R(*ci*) – 23	92 *E*	2	13	21	.	..	40	20	5	.	..
R(*ci*) – 29	94 *A*	21	11	28	4	..	11	26	21	2	..
R(*ci*) – 32	92 *E*	38	33	55	9	..	15	17	58	6	..

* ey^D = eyeless-dominant; chromosome IV.

† *N* = uninterrupted distal section of 4th vein; 0 = uninterrupted but thinned section; 1, 2 = different progressive degrees of interruption; 3 = absence of whole section.

‡ The *R*(*ci*) alleles are numbered in chronological order of their discovery.

be stated that the maximum of the frequency distributions of venation classes in *R*(*ci*)/*ci* are shifted to the next, more extreme class or even farther. Table 2 (right column) shows that the five position alleles differ in the degree to which they cause the expression of the *ci* phenotype in the combination *R*(*ci*)/*ci*. It is possible to distinguish two groups of *R*(*ci*) alleles, one leading to a distribution of phenotypes around a mean located between Classes 1 and 2, and another with the more extreme mean between Classes 2 and 3. Comparing the degree of effect of the *R*(*ci*) alleles in *R*(*ci*)/+ (table 1) with *R*(*ci*)/*ci* (table 3), it is seen that a strong effect in the former constitution is paralleled by a strong effect in the latter and vice versa.

Heterozygotes between Two Position Alleles R(+) and R(ci).—A translocation N^{264-20} between the first and fourth chromosome, produced by x-radiation of a fly with normal alleles of *ci*, was obtained through the

courtesy of Dr. M. Demerec.[8] Females heterozygous for this translocation show a Notch (N) phenotype. They were crossed to *ci* males. Among the offspring those which were heterozygous for the translocation and *ci* exhibited a typical "Dubinin effect": Their phenotype showed *ci* venation with a maximum at Class 2 (table 3). Thus, the N-translocation may be labeled as possessing a position allele $R(+)$.

Females of the constitution $R(+)/ci$ were mated to males which carried one of the five tested $R(ci)$ alleles and, as a marker for their non-translocated fourth chromosome, the dominant ey^D. The non-ey^D female offspring of these crosses were either $R(ci)/ci$ (table 2, right column) or $R(+)/R(ci)$ (table 2, left column). In all five cases the $R(+)/R(ci)$ flies were more normal than their $R(ci)/ci$ sibs (right column) or than $R(+)/ci$ flies (table 3). Indeed, three different types of $R(+)/R(ci)$ flies were nearly

TABLE 2

F_1 NON-ey^D ♀♀ OF *y* $N^{264\text{-}20}$ B^iB^i; $R(+)/ci\ ey^R \times R(ci)/ey^D$ 26°C.

	$R(+)/R(ci)$					$R(ci)/ci\ eyR$				
	N	0	1	2	3	*N*	0	1	2	3
$R(ci)$ – 8	37	12	24	17	1	3	7	27	18	1
$R(ci)$ – 48	35	12	31	29	4	.	8	45	62	6
$R(ci)$ – 23	15	1	..	..	.	.	1	1	12	5
$R(ci)$ – 29	48	1	..	4	.	.	.	4	16	20
$R(ci)$ – 32	50	..	..	..	.	.	.	3	16	25

TABLE 3

F_1 ♀♀ OF *y* $N^{264\text{-}20}$ B^iB^i; $R(+)/y\ Hw\ m^2\ g^4\ dl$-49 * × $ci\ ey^R/ci\ ey^R$ 26°C.

$R(+)/ci\ eyR$				$Hw/ci\ eyR$	
N	0	1	2	3	*N*	0-3
..	4	40	186	27	275	..

* *y*, *Hw*, B^iB^i, m^2 and g^4 are sex-linked factors which segregate together as a unit due to the presence of *dl*-49, an inversion, and of $N^{264\text{-}20}$.

completely normal. Moreover, the curious relation is apparent that those $R(ci)$ alleles which cause an extreme *ci* phenotype in either combination $R(ci)/+$ or $R(ci)/ci$ produce the least extreme expression in $R(ci)/R(+)$.

It will be necessary to test different $R(+)$ alleles with the $R(ci)$ alleles recorded in table 2, and with others, in order to find out how general are these unexpected results. We have crossed three of the $R(ci)$ alleles used in combination with the $R(+),N^{264\text{-}20}$, with another $R(+),w^{258\text{-}18}$. This latter translocation does not show abnormal "Notch" wing characters as does $N^{264\text{-}20}$. Nevertheless, the $R(+),w^{258\text{-}18}$, gave equivalent results in combinations with $R(ci)$, indicating that the approach to normal, non-*ci* venation in $R(+)/R(ci)$ is independent of the specific phenotype caused by Notch.[9]

Discussion.—When Dubinin and Siderov[2] found that $R(+)$/haplo-IV

and $R(+)/R(+)$ produce normal phenotypes while $R(+)/ci$ flies have interrupted veins, it seemed that such relations between alleles were unique for the position effect. It has since been shown (Stern 1943)[10] that the action of *ci* alleles may be described in terms of combining ability, c, with a cellular substrate, S, and efficiency, e, in transforming S into a product, P, effective in leading toward a normal phenotype. With the help of these concepts the Dubinin effect can be understood if it is assumed that there is a sufficient amount of substrate and that the $R(+)$ allele has sufficient combining power and efficiency in homo- or hemizygous constitution to cause normality; that in the heterozygote $R(+)/ci$ there is competition for S between the $R(+)$ and *ci* alleles, and that the efficiency of *ci* is less than that of $R(+)$, and hence the amount of P formed is below normal. Similarly the phenotypic effect of $R(ci)$ in the combination $R(ci)/+$ can be understood as the outcome of competition between $R(ci)$

TABLE 4

POSSIBLE CHANGES OF GENIC ATTRIBUTES

	COMBINING POWER c	EFFICIENCY e
1	Unchanged	Lower
2	Unchanged	Higher
3	Lower	Unchanged
4	Higher	Unchanged
5	Lower	Lower
6	Lower	Higher
7	Higher	Lower
8	Higher	Higher

and + in which the former deprives + of S and transforms S into P at a low efficiency. If the effects of the changed position of the + and *ci* allele are described in terms of dominance and recessiveness, the change from + to $R(+)$ as seen in $R(+)/ci$ versus $+/ci$ would be called a *decrease* of dominance of the + allele while the change from *ci* to $R(ci)$, as seen in $R(ci)/+$ versus $ci/+$ would be an *increase* of dominance of the *ci* allele. The interpretation of position effects in terms of competition resolves the apparent paradox.

In the preceding paragraph the interaction of the various alleles has been considered separately for certain allelic combinations. This led to the recognition of competition between alleles, and of the relative efficiencies of + as compared with *ci*, of $R(+)$ as compared with *ci*, and of + as compared with $R(ci)$. It is now appropriate to ask in which way the position allele $R(+)$ differs from the original allele +, and $R(ci)$ from *ci*. If the amount of available substrate S is the same for the gene in its altered as in its original position, then all changes in effect due to new positions of alleles will be the result of changes in the combining power,

c, or of the efficiency, e, or of both. We shall begin our analysis with this assumption of constancy of S. Variability of S due to changed position of alleles will be considered later. Since it is possible *a priori* that either c or e may remain unchanged, or be increased or decreased there are eight combinations possible in which at least one of the two properties is altered (table 4). Considering that there exists competition in the heterozygote $+/ci$ between $+$ and ci the decreased effect of $R(+)/ci$ could be brought about by any one of the following changes of the $R(+)$ allele as compared with $+$: (1), (3), (5), (6) and (7). On the other hand, the decreased effect of $R(ci)/+$ could be produced by any one of the following changes of the $R(ci)$ allele as compared with *ci:* (1), (4), (5) and (7). Since it is reasonable to assume that the type of change caused by the altered position is the same, in general, for $R(+)$ and $R(ci)$ three possibilities which account for changes in only one of either $R(+)$ or $R(ci)$, but not in both, can be eliminated, namely (3), (4) and (6). This leaves (1), (5) and (7). These three types of changes all agree in that they involve a lower efficiency of the position allele as compared with the original allele. They differ in the type of c attribute which may be unchanged, lower or higher than in the original allele. No information is available at present which permits a decision as to which of these three possibilities is actually realized. Any one of them is not only compatible with the effect of $R(+)/ ci$ and $R(ci)/+$ but also with the lowered effect of $R(ci)/ci$ as compared with ci/ci. Competition, with low efficiency of a position allele, accounts not only for the "dominant" mutant effect of $R(ci)$ but may provide a general explanation for the occurrence of dominant effects in loci which otherwise have produced recessive mutants only (e.g., Plum).

It will elucidate the problem further to discuss briefly in what ways a new position may cause a change in the effect of a gene.[11] If genic action consists of a primary interaction with a substrate, S, leading to a product, P, one may conceive of this interaction as being composed of two separate steps. The first would consist of a reaction leading to a gene-substrate association, the second to restoration of the gene with release of P. The term combining power would apply specifically to the first reaction and be equivalent to an association constant. The term efficiency might be equivalent to the velocity constant which characterizes the second reaction, provided the gene products P of the different alleles are alike or at least equivalent in their effect. If the gene products are different and have different values in leading toward a given phenotype, then the efficiency, e, of an allele is a function of both the velocity constant and of the effectiveness of its specific P. The deduction—made under the unproved assumption of constancy of S—that the observed position effects at the ci locus involve a lowered efficiency, localizes these effects on the second step of the gene-substrate interaction. Whether changes in the first step of this re-

action are also involved remains undecided. This analysis of the position effect, however, leaves open the question as to the primary change responsible for the changed reaction. The primary change may be an alteration of actual genic structure, or some kind of alteration due to unusual synaptic forces (Muller, 1935).[12] It may even not involve any alteration of the gene itself but may be due either to a changed local environment under which the reactions must proceed or due to a changed amount of appropriately localized and accessible substrate. If in the new position less substrate were available than originally, a lowered total effect would result in the heterozygote of a position allele and the allele in the normal chromosome. By making, in this case, the unjustified assumption of an unchanged amount of S the lowered effect would be mistakenly interpreted as due to changes in the c and e attributes. An example may clarify this point. Be it assumed that the $R(+)$ and $R(ci)$ positions involve a reduction of available S. Since, in $R(+)/ci$, $R(+)$ and ci share in S the actual amount of S available not only to $R(+)$ but also to ci might be diminished. Similarly, in $R(ci)/+$, the amount of S available not only to $R(ci)$ but also to $+$ might be diminished. If, without knowledge of the decrease in S, the amount of the latter, as well as the c and e properties of the normally located alleles ci and $+$ were regarded as unchanged, then the change in total P produced by $R(+)/ci$ and $R(ci)/+$ would appear as caused by a lowered e of $R(+)$ and $R(ci)$ (with or without change of their c). In the absence of information as to the amount of available S in heterozygotes for position alleles as compared with typical heterozygotes, we may summarize our analysis by stating that competition between the alleles is involved in the case of position effects of the ci locus as it is in typical heterozygotes but that the primary cause or causes of the difference in effect of position heterozygotes as compared with typical heterozygotes remains an open problem. Such a primary factor may either be a diminished amount of available substrate, a decreased efficiency of the position alleles, or possibly a combination of both.

While the proposed interpretation in terms of competition of the position alleles seems to unify most facts concerning gene action of typical and position alleles, it is unsuccessful without additional hypotheses in accounting for the effect of the combination $R(+)/R(ci)$. Since both $R(+)/ci$ and $R(ci)/+$ cause an effect less normal than $+/ci$, it was expected that $R(+)/R(ci)$ would be still less normal. Instead, this constitution causes a shift, in some cases nearly complete, toward normality. Possibly this fact is related to the type of somatic pairing occurring in heterozygotes for two translocations as compared to heterozygotes for only one translocation. No satisfactory interpretation, however, can be proposed at present.[13] It may be added that an apparently similar case has recently been described in an abstract by Oliver (1943),[14] though his inter-

pretation differs from the one given for *ci*. He found that the "dominant" eye color Punch which arose in connection with a translocation becomes "recessive" when combined with another translocation in the homologous chromosome which carries a normal allele.

Incidentally, the data reported furnish a unique example of the reality of "premutation," or "directed mutation" as postulated frequently in the early genetic discussions of evolutionary phenomena. The three "alleles" +, *ci* and *R(ci)* according to their effect may be described as dominant normal, recessive mutant and dominant mutant. A dominant mutant allele at the *ci* locus has only twice been found to be due to a single-step mutation from a normal allele. Our data show that such dominant mutant "alleles" can be easily produced by a step involving change of the effect of the recessive mutant *ci* which itself is removed by one mutational step from +.

Summary.—In *D. melanogaster* five translocations between the third and fourth chromosome, carrying the mutant allele *ci*, were studied. In combination with an unbroken fourth chromosome containing a normal allele they produce, in varying degrees, *ci* phenotypes in contrast to the typical heterozygote +/*ci* which appears nearly normal. Combined with *ci* in an unbroken fourth chromosome they lead, in varying degrees, to more extreme *ci* phenotypes than *ci/ci*. The three translocations which cause the greatest deviation from normality in combination with + also do so in combination with *ci*. A translocation between chromosomes 1 and 4 derived from a stock containing a normal allele of *ci* produces a striking *ci* phenotype if combined with *ci* in an unbroken chromosome ("Dubinin effect"). If this translocation instead is combined with *ci*, as carried in any one of the five translocations, a shift of phenotypes toward normality occurs. Those translocations which cause the least normal phenotypes in combination with + and *ci* are most effective in causing a shift toward normality when combined with the +-carrying translocation.

Many, but not all, facts reported can be interpreted in terms of concepts regarding genic action which have recently been proposed.[10] Other facts seem to involve additional phenomena. The position effects in heterozygotes appear as competition between the alleles in the normal and changed position.

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[1] An abstract had been submitted to the Genetics Society for the 1942 meeting; see Stern, C., *Genetics*, **28**, 92–93 (1943).

[2] Dubinin, N. P., and Siderov, B. N., *Am. Nat.*, **68**, 377–381 (1934).

[3] We wish to acknowledge the kindness of Dr. S. W. Warren, Professor of Radiology, The University of Rochester School of Medicine and Dentistry, who carried out the radiations.

[4] ey^R, a recessive allele of eyeless, served as a marker gene for non-radiated fourth chromosomes. It is without influence on the expression of *ci*. Except in the tables, reference to ey^R will be omitted.

[5] The normal allele was the $+^C$ allele of the *ci* locus, see Stern, C., and Schaeffer, E. W., *Proc. Nat. Acad. Sci.*, **29**, 361–367 (1943).

[6] After completion of the manuscript of the present communication a paper by Siderov, B. H., *Compt. rend. acad. sci. U.R.S.S.*, **31**, 390–392 (1941), has come to attention in which similar effects of *R(ci)* alleles in combination with + are described.

[7] Khvostova, W. W., and Gavrilova, A. A., *Biol. Zhurnal.*, **4**, 905–916 (1935).

[8] "Salivary chromosomes (Sutton) show break in *X* at 3 C, with deficiency of 3C 5–7; and break in 4 at 101*F*," Bridges and Brehme, "The Mutants of *Drosophila melanogaster*," *Carnegie Inst. Wash. Publ.*, **552** (1944).

[9] In the paper of Siderov referred to in footnote 8 a further remarkable fact is reported. Two translocations between the third chromosome and a fourth chromosome which carries a + allele but which do not give a Dubinin effect in combination with *ci*, shift the phenotype toward normality if combined with *R(ci)*.

[10] Stern, C., *Genetics*, **28**, 441–475 (1943).

[11] Grateful acknowledgment is made to Dr. D. R. Goddard for clarification of some of the concepts involved in this discussion.

[12] Muller, H. J., *Summ. Commun. XV int. physiol. Congr.* (Leningr.-Mosc.), pp. 286–289 (1935); and *Proc. 15th int. physiol. Congr.* (Leningr.-Mosc.), pp. 587–589 (1938).

[13] If it were true that the new positions imply a reduction of *S* at the *ci* locus and if heterozygotes between normally located and position alleles frequently undergo somatic pairing while compounds between *R(+)* and *R(ci)* rarely pair, then competition might take place between the paired alleles of heterozygotes, while no competition might occur between the separated alleles *R(+)* and *R(ci)*. The lack of competition might then permit a shift toward normality. The assumptions made are liable to experimental test.

[14] Oliver, C. P., *Anat. Rec.*, **87**, 33 (1943).

COMPARISON OF A DEGENERATE FORM OF EINSTEIN'S WITH BIRKHOFF'S THEORY OF GRAVITATION

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Whereas electric charge is not universally proportional to the inertial mass of bodies their gravity is. This fundamental fact, supported both by daily experience and the most refined experiments, led Einstein to the conception that inertia and gravitation are one (principle of equivalence) and thus to his theory of general relativity. The main reason for my and many others' belief in that theory is the radical explanation it affords for the fact just mentioned. Any theory which breaks up the unity of inertia and gravitation, as Birkhoff's recent theory of gravitation in a flat world[1] does, throws us back into the position before Einstein where we had to