# The Phenomenon of Position Effect

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## I. INTRODUCTION AND CLASSIFICATION OF POSITION EFFECTS

That the effect of a gene may be dependent upon its position with respect to neighboring genes is now a well-established fact in *Drosophila melanogaster* and one which has recently been demonstrated in a convincing way in *Oenothera blandina* (Catcheside, 1947a). This phenomenon of position effect (Sturtevant, 1925) has long been recognized as a fundamental problem in genetic theory and one which should throw light on the organization of the chromosomes as well as on the primary reactions of specific genes. Notable advances have been made by the accumulation of many examples of position effect in Drosophila and by the discovery of some of the necessary conditions for its detection. At the same time a considerable body of evidence bearing on this subject has yet to be incorporated into a consistent theory. In this review a

TABLE 1

Loci, normally located in euchromatin, whose normal alleles exhibit a V-type position effect when abnormally set next to heterochromatin. Loci marked by (*) are known only by dominant mutants which are associated with small duplications; variegation of these genes in the examples given is probable but not certain.

| *Mutant* | *Description* | *Symbol* | *Locus* | *Example of Rearrangement* | *References* |
|---|---|---|---|---|---|
| Yellow | Bristle-body-color. | y | X- 0.0 | $y^{29}$ | Noujdin (1935, 1936, 1944) |
| Achaete | Missing hair and bristle pattern of "ac" type. | ac | - 0.0 | $sc^{8}$ | Noujdin (1935, 1944); Crew and Lamy (1940) |
| *Hairy-wing | Extra hairs and bristles of "ac" and "sc" type. | Hw | - 0.0 | $sc^{8}$ | Alikhanian (1938); Crew and Lamy (1940); Bridges and Brehme (1944) |
| Scute | Missing hair and bristle pattern of "sc" type. | sc | - 0.0 | $sc^{8}$ | Bridges and Brehme (1944) |
| Lethal (1) 7 | Lethal; light eye-color as a mosaic. | 1(1)7 | - 0.3 | $Dp(1;f)X^{e2}$ | Bridges and Brehme (1944) |
| White | Eye-color. | w | - 1.5 | $w^{m4}$ | Muller (1930); Gowen and Gay (1934); Demerec and Slizynska (1937) |
| Roughest | Roughened eye. | rst | - 1.7 | $rst^{2}$ | Grüneberg (1937); Kaufmann (1942); Demerec and Slizynska (1937) |
| Facet | Rough eye; nicked wing. | fa | - 3.0 | $N^{264-57}$ | Demerec (1940, 1941a) |
| Split | Rough eye; extra bristles. | spl | - 3.0 | $w^{258-21}$ | Schultz (1941a) |
| Diminutive | Bristles, body, small. | dm | - 4.6 | $N^{264-52}$ | Demerec (1940, 1941a) |
| Echinus | Enlarged facets. | ec | - 5.5 | $N^{264-53}$ | Demerec (1940, 1941a) |
| Bifid | Fused venation. | bi | - 6.9 | $N^{264-52}$ | Demerec (1940, 1941a) |
| Rugose | Rough eyes. | rg | - 11.0 | $N^{264-85}$ | Demerec (1940) |
| Curlex | Curled wings. | cx | - 13.6 | $N^{264-85}$ | Demerec (1940) |
| Crossveinless | Missing crossveins. | cv | - 13.7 | $N^{264-85}$ | Demerec (1940) |
| Roughex | Small, rough eyes. | rux | - 15.0 | $N^{264-85}$ | Demerec (1940) |
| Vesiculated | Blistered wings. | vs | - 16.3 | $N^{264-85}$ | Demerec (1940) |

| | | | | | |
|---|---|---|---|---|---|
| Forked | Twisted bristles. | f | - 56.7 | $f^{B13}$ | Belgovsky (1938, 1944, 1946); Noujdin (1946a) |
| *Bar | Small eye. | B | X - 57.0 | $B^{M1}$ | Belgovsky (1938); Dubinin and Volotov (1940); Sutton (1943a) |
| Aristaless | Short aristae. | al | 2 0.0 | $al^{V1}$ | Lewis (1945) |
| Asteroid | Small, rough eyes. | ast | - 1.3 | $ast^{V1}$ | Lewis (1945) |
| Brown | Eye-color. | bw | - 104.5 | Pm | Glass (1933); Schultz and Dobzhansky (1934); Dubinin and Heptner (1935) |
| Minus | Bristles, body, small. | mi | - 104.7 | Pm | Schultz and Dobzhansky (1934) |
| Abbreviated | Bristles, body, small. | abb | 2 - 105.5 | Pm | Schultz and Dobzhansky (1934) |
| Hairy | Extra hairs. | h | 3 - 26.5 | T(3;4)684 | Dubinin and Sidorov (1935) |
| Curled | Curled-wing; upturned bristles. | cu | 3- 50.0 | $T(3;4)D1^{7P}$ | Panshin (1935) |

survey of major developments in the field will be presented, together with a discussion of the properties and possible mechanisms of position effect. For much of the early literature on this subject the reader is referred to the review of Dobzhansky (1936).

It has become increasingly evident that there exist at least two distinct types of position effect in Drosophila, and it may be questioned whether they are in fact causally related to one another. In one type, the change in gene action associated with a change in gene position is subject to wide and frequent statistical fluctuations, often among related cells in the same individual, and it thus results in a type of somatic mosaicism. In such cases the associated rearrangement invariably appears to involve the euchromatic and heterochromatic regions of the chromosomes (Schultz, 1936). The phenomenon of somatic instability in gene action arising in association with such rearrangements has been designated "variegation" (Schultz, *loc. cit.*), and changes of this type have been referred to as "eversporting displacements" (Muller, 1930). It will be convenient to refer to this category of effects as the variegated- or V-type position effects. To avoid circumlocution, the expression "variegation of a gene" will be used to express the variegation of a phenotype controlled by that gene. The V-type appear to constitute the bulk of position effects known in Drosophila, and although the cytological picture is lacking, position effects so far detected in Oenothera are probably of this kind. A rather small group of position effects is known in which the change in gene action is of a somatically stable type as with most changes within the gene itself. When a rearrangement is associated with such cases it often involves the wholly euchromatic regions of the chromosomes. This group will be referred to here as the stable- or S-type position effects. Fortunately, several other properties serve to distinguish these two categories of position effect in those cases where a distinction between instability and stability in gene action is not readily made. The V-type position effects are considered first inasmuch as they have been more extensively studied.

## II. Variegation of Genes Located in Euchromatin

### 1. *The Variegated Phenotype*

A large number of genes in Drosophila are now known to exhibit a somatically mosaic phenotype when brought into association with heterochromatin (Table 1.). Although variegation does not appear to be restricted to any particular type of gene (except in so far as its detection depends on an autonomous gene action), relatively few genes have effects which make them suitable for a developmental study of the variegated

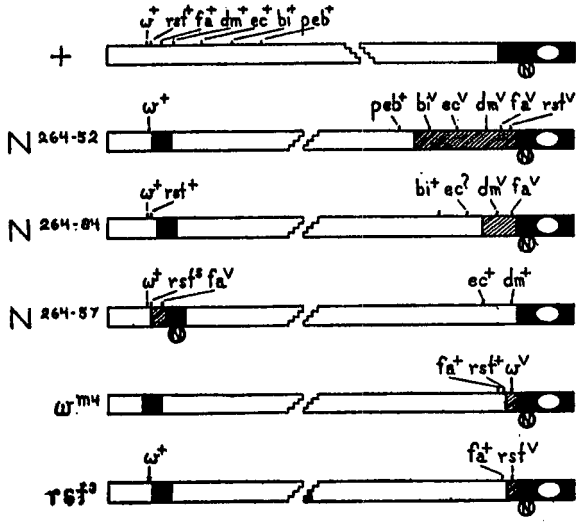


FIG. 1

Examples (diagrammatic) of X-chromosome inversions associated with variegation of genes (see Table 1 and Fig. 4), with reference to the normal chromosome at top of figure. Legend: black = heterochromatin of the X chromosome; unshaded areas = euchromatin; shaded areas = the extent of spreading of the variegated effect; N = nucleolar organizing region; V = variegated; S = stable; + = non-variegated or wild-type.

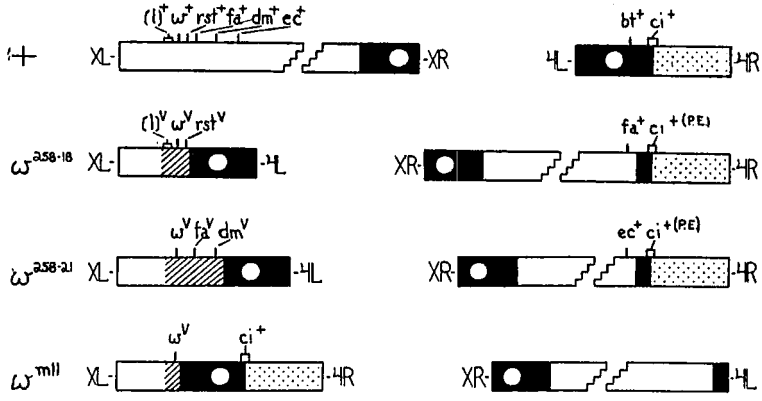


FIG. 2

Examples of translocation between the X and fourth chromosomes detected as white-variegated types (diagrammatic). Legend: black = heterochromatin of X or 4; unshaded = euchromatin of X; stippled = euchromatin of 4; shaded = extent of spreading-effect of the variegation process; (P. E.) = a position effect of the cubitus interruptus gene.

phenotype. Of these, the most widely studied example is the normal allele of the white gene ($w^+$), variegation of which can be detected as a color change over the range from red to white in the 850 or so ommatidia, or "facets," of the compound eye. Examples of rearrangements of this allele having a V-type position effect are shown diagrammatically in Figs. 1 and 2. Although these types are customarily designated as mutant alleles of the white gene, for example, $w^{m4}$, for white-mottled-4, it is convenient to introduce a more generalized notation which expresses at once the presence of a rearrangement (R) and the allele present at the time the rearrangement was produced. Such a terminology, introduced by Stern and Heidenthal (1944) for rearrangements having a position effect of the cubitus interruptus (*ci*) gene in the fourth chromosome (Section III.2), will be extended here to designate rearrangements having a V-type position effect. Thus R($w^+$) will be used to designate a rearrangement associated with variegation of the normal allele of the white gene; and R in general has, as in the case of R($ci^+$) types, the significance of a euchromatic-heterochromatic rearrangement.

Descriptions of the phenotypes associated with R($w^+$) types have been given in some detail by Muller (1930), Gowen and Gay (1934) and Demerec and Slizynska (1937). A specific effect on the white gene is clearly demonstrated by the fact that the heterozygote between R($w^+$) and a chromosome bearing the mutant white allele ($w$) has a variegated eye color, whereas R($w^+$) / + heterozygotes have normal red eyes. When viable, R($w^+$) homozygotes and hemizygotes likewise have a variegated phenotype. The variegation shows an extraordinary range of variability depending on the particular R($w^+$) type being considered and the genetic and environmental background (Section II.5). The variegated eye may have a red background color with scattered lighter patches; a white or light background with scattered red or dark facets; and frequently it is of an intermediate color such as cream, or pink, with darker and/or lighter patches present. Facets of intermediate colors have been interpreted on the basis of an all-or-none change by assuming that a mixture of red and white cells are present in the complex of pigment cells which make up an individual facet (Panshin, 1938). Schultz (Morgan *et al.*, 1937) has argued for an all-or-none effect in the case of white-variegation in the Malphigian tubules, where isolated single white cells can be detected in an otherwise yellow tubule. Demerec and Slizynska (1937) assume that gene mutation occurs and that mutation to an intermediate allele of the white series may produce the intermediate background colors. Another special consideration is the light or white background in which a few scattered facets of darker pigmentation may occur (figured by Muller, 1930). These

dark spots have been interpreted as cases in which residual normal cells remain (Schultz, 1941a) and as evidence of reverse mutation (Demerec and Slizynska, 1937); in the latter case, a less restrictive assumption would be that a recovery in gene function may occur, since there is no proof that a process akin to gene mutation is involved (Section II.7). As Gowen and Gay (*loc. cit.*) have found, variegation in an R($w^+$) heterozygote having an intermediate allele of the white series in the normal chromosome, does not give rise to facets of a lighter color that those of the heterozygote between that allele and the white mutant.

Variegation of genes affecting the color, structure, or presence of bristles is a more instructive effect than eye-color variegation since it is measurable in terms of specific cells. Noujdin (1938) has noted that (*a*) variegation of the yellow (*y*) gene in an R($y^+$) type (the $y^{sp}$ inversion of Patterson) may result in bristle colors ranging from black (wild-type) through dark brown and light brown shades to full yellow; and (*b*) the degree to which a bristle may manifest a forked effect, due to variegation of the forked (*f*) gene in certain R($f^+$) types of Belgovsky is also variable. Thus the possibility is open that the variegation process may be subject to variation within a given cell as well as between cells.

Several cases are known in which variegation behaves as a dominant character. Thus variegation, presumably for the facet gene ($fa^+$), results in a variable dominant phenotype of the Notch-type (Muller, 1930). Examples of R($fa^+$) alleles of this type are the Notch-variegated types of Demerec (1941a) shown in Fig. 1. Since the Notch phenotype is known to result from a deficiency for the facet gene, variegation in these cases is consistent with the assumption that inactivation of the gene, or its product, occurs in the V-type position effects. Apparent exceptions to this rule are found in certain other dominant variegated-types, notably, in rearrangements of the normal allele of the brown gene ($bw^+$) to heterochromatin. Thus R($bw^+$) / + shows mottled brown and red facets, while "allelism" with the brown gene is indicated by the fact that R($bw^+$) / *bw* has an almost homogeneous brown eye color (Glass, 1933; and Schultz and Dobzhansky, 1934). Here, as the latter workers found, there is evidence that a deficiency for the region containing the brown gene does not give a dominant brown phenotype nor does the presence of two doses of the $bw^+$ allele suppress the dominance. Ephrussi and Sutton (1944) have interpreted the dominance as an effect exerted by the rearrangement on the $bw^+$ allele in the normal as well as in the rearranged chromosome. On this basis an R(*bw*) / + fly should also show dominant brown-variegation as indeed was the case in a rearrangement of the R(*bw*) type obtained by Moore by x-raying the mutant brown (Glass, 1933). Hinton's discovery of an extreme dominant brown mu-

tant, $bw^D$, which Schultz has interpreted as a one-band duplication in the salivary gland chromosomes (cited in Bridges and Brehme, 1944), indicates that the dominant effect does not require a major chromosomal rearrangement for its production, but rather that it may be a potential property of the $bw^+$ locus or of a neighboring pseudo-allelic locus as in the Star and asteroid mutants (Section IV.1). A hairy-wing effect, probably a V-type position effect, is conspicuously dominant in scute-8 heterozygotes (see Fig. 3). Here again the phenotype is analogous to that of a small duplication (for the 1 B1-2 double band, Fig. 4), namely, hairy-wing, or to that of duplications for the tip of the X chromosome including this locus, but not to deficiencies for this region (Demerec and Hoover, 1939). A model for these dominant effects may be made by assuming that inactivation of one gene leads to an accumulation of the precursor substance normally utilized by that gene and that this excess results in the dominant change. This would reconcile dominant hairy-wing- and dominant brown-variegations with the other V-type position effects as being essentially equivalent to a process of inactivation of the gene or its products. To explain the failure of a deficiency to produce the dominant phenotype would require the assumption that the loss involves also the locus of the gene producing the substance required by the gene in question. A mechanism involving competition of two such linked genes for the same substrate would be equally applicable. Competition between a gene in the normal chromosome and its allele in the rearranged chromosome has been proposed as a model for such dominant effects as that of R($bw^+$) by Stern and Heidenthal (1944), such that the rearranged allele retains an ability to compete with the allele in the normal chromosome but owing to its new position is unable to function as efficiently as the latter (Section III.2).

### 2. *Proofs of the Variegated-Type Position Effects*

Dubinin and Sidorov (1935) obtained direct proof of the position effect phenomenon in a study of a translocation between the third and fourth chromosomes associated with a variable change in action of the hairy ($h$) gene in the left arm of chromosome 3. The heterozygote between the hairy mutant and this rearrangement showed a range of one to eleven extra hairs on the scutellum in contrast to "several dozens" in homozygotes for hairy, and none in wild-type. This along with the salivary gland chromosome analysis showing a rearrangement involving the proximal or heterochromatic region of chromosome 4 serves to identify this as a V-type position effect. As the result of crossing over between the locus of hairy and the breakpoint of the rearrangement, a translocated chromosome carrying the mutant hairy gene, that is, R($h$),

was derived from R($h^+$) / $h$ females. The insertion of an $h^+$ allele, derived from a normal chromosome, was obtained from R($h$) / + females, as the result of the same type of crossing over. The newly introduced $h^+$ allele was found to acquire the same instability in its somatic action as that found with the $h^+$ allele in the original translocation. Since precisely similar pairing relationships are expected in R($h$) / + and R(+) / $h$, and since the former was reported to be associated with a wild-type phenotype and the latter with a variable, hairy phenotype, the position effect cannot in this case depend solely on structural heterozygosity. The fact that only R($h^+$) / $h$ departed from normality may be taken to indicate that $h^+$ is altered only when in the rearranged chromosome. Parallel experiments with equivalent results were conducted by Panshin (1935) with a translocation between the third and fourth chromosomes associated with an effect on the normal allele of the curled gene ($cu^+$). Variegation was in evidence in the sense that a population of R($cu^+$) / $cu$ flies showed a wide range of expression of the curled-wing phenotype; in contrast to the constant curled-wing and bristle effect of the $cu$ homozygote. In this case it was reported that 0.8% crossing over was observed between the locus of curled and the breakpoint of the rearrangement; and that five cases of R($cu$) types, representing the insertion of the mutant $cu$ gene into the rearrangement, were recovered. From each of these, R($cu^+$) types, representing the insertion by crossing over of $cu^+$ from a normal chromosome, were obtained. Using a rough grading system Panshin showed that the "new" R($cu^+$) / $cu$ had the same variable curled phenotype as the heterozygotes between the curled mutant and the original translocation.

Grüneberg (1937) reported another type of proof of the position effect as the result of a discovery of a reversal of the rough-eyed phenotype associated with the X chromosome inversion, $rst^3$ (Fig. 1). Genetic analysis and salivary gland chromosome studies (Emmens, 1937) indicated that this change was accompanied by reinversion of the rearrangement, which, as far as was determined, restored the original gene order. A more critical test, the phenotype in the XO male, was not employed (Section II.5). Although Kaufmann (1942) was unable to secure reinversion of $rst^3$ following x-ray treatment of $rst^3$ males, his discovery that induced "reverse mutations" of $rst^3$ are accompanied by new rearrangments (Section II.6) usually returning the roughest gene to a euchromatic position, provides additional evidence that the normal allele, $rst^+$ is still present in the rearranged chromosome.

A strong indirect proof of the V-type position effect can be made out in such cases as the white locus where direct proof is still lacking. Here, over 35 instances of x-ray white-mottled types have been analyzed

by the salivary gland chromosome method as the result of work by Schultz (1936), Sacharov (1936), and particularly by Demerec (1941a) and coworkers. Without exception each of these cases was associated with a rearrangement bringing one of the heterochromatic regions and the white locus into close proximity. Attempts by Griffen and Stone (1940) to explain this association with heterochromatin as the sole result of a relatively high breakage frequency in the heterochromatic and white regions are not supported by (*a*) Kaufmann's (1946) finding that rearrangements involving the 3 C, or white region more often involved euchromatin than heterochromatin and, more generally, that regions showing relatively high breakage frequencies, such as 3 C, do not recombine preferentially with heterochromatin; nor (*b*) Demerec's (1941a) finding that rearrangements involving a break near the white locus and another in euchromatin were either unassociated with a change at the white locus or carried a stable mutant allele or an S-type position effect of that gene.

### 3. *The Specificity of the Heterochromatin Association*

Demerec (1941a) has shown that variegation involving the white gene may be caused by the association of $w^+$ with heterochromatin derived from the X chromosome or from any one of the major arms of the autosomes. Heterochromatin from the short arm of the fourth chromosome also appears to be effective in inducing variegation of this gene as first noted by Panshin in white-mottled-11 (Fig. 2). In two cases heterochromatin from the tip of the fourth chromosome was effective, but otherwise breaks were confined to the proximal heterochromatic regions of the chromosome arm involved. Demerec showed that differences exist within the heterochromatic region of a given chromosome arm in its capacity to induce variegation. Thus, not every rearrangement which brings heterochromatin into the immediate vicinity of a gene causes variegation of that gene as may be seen in the Notch variegated types, $N^{264\text{-}52}$ and $N^{264\text{-}57}$, in which variegation of the diminutive (*dm*) gene occurs in the former, but not the latter where the gene is actually closer to heterochromatin.

Breakages in the heterochromatin of the X chromosome are illustrative since they can be differentiated with reference to (*a*) a nucleolar organizing region (Kaufmann, 1942, 1944), (*b*) the so-called "block A" region (Muller *et al.*, 1937), which constitutes a sizable portion of the heterochromatic region of the X chromosome in dividing cells, and (*c*) a region containing the bobbed gene. This differentiation is shown diagrammatically in Figs. 1 and 3, for the normal chromosome, and for certain inverted sequences associated with V-type position effects. Variega-

tion has been found to occur whether or not the affected gene is next to (*a*) the centromere (*cf.*, the facet gene in the above-mentioned Notch inversions); (*b*) the nucleolar organizing region (*cf.*, the white gene in $w^{m4}$ and the scute gene in $sc^8$); and (*c*) block A or the bobbed gene (*cf.*, the yellow and scute genes in $sc^8$). Although no wholly consistent rules are in evidence, variegation is, as Demerec (1940, 1941a) has discussed, more extreme (a higher proportion of mutant than normal tissue) when the effected

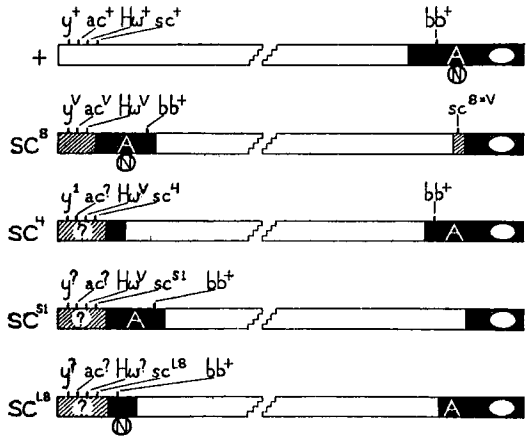


FIG. 3

Examples of X-chromosome inversions associated with variegation of genes in the yellow-scute region with reference to the normal chromosome. (See Fig. 1 for legend; A = Block A; and see Fig. 4 for cytological details). Diagrammatic.

gene is near the centromere; or, at least the variegation process exerts an effect over a greater distance in this case (Section II.4). Panshin (1938) found evidence that the greater the amount of heterochromatin brought next to the white gene the more extreme the variegation (Section II.6). Evidently, highly specific differences also exist within a given heterochromatic region in its capacity to induce variegation, as in the case of $N^{264\text{-}57}$ (or $rst^3$) *vs.* $N^{264\text{-}52}$, cited above. It may be concluded that the establishment of a close association between heterochromatin and a gene normally lying in the wholly euchromatic region is a necessary but not always a sufficient condition for the production of a V-type position effect of that gene.

### 4. *The Spreading Effect*

One of the most remarkable properties of the variegation process is the frequent tendency for several genes in the vicinity of the point of rearrangement of euchromatin and heterochromatin to be affected.

Muller (1930) first noted this effect in a white-mottled type ($w^{m1}$) which had in fact been detected originally as a Notch mutation; it was observed that the extent of white-mottling was in this case directly correlated with the extent of Notch expression. Gowen and Gay (1934) showed that such an effect must be limited since no change in action of genes remote from the white locus occurred, thus ruling out the possibility that a type of unstable translocation was involved (Patterson, 1932b). Demerec (1940, 1941a) has made extensive studies of this process. In the inversion, $N^{264\text{-}52}$ (Fig. 1), variegation for 5 genes was observed and was correlated with the rearrangement of these genes to the centromere region of the X chromosome. In this case the effect was observed to spread at least as far as the bifid locus, located in the salivary gland chromosomes at least 50 bands removed from the point of rearrangement.

A unique opportunity for studying this "spreading effect" is presented by certain white-mottled types having an associated change at the roughest locus and/or the split locus. Demerec and Slizynska (1937) studied a white-mottled type, $w^{258\text{-}18}$, which was accompanied by variegation of the roughest gene lying immediately to the right of the white locus. Only three general types of facets were found in the eyes of homozygotes or hemizygotes for this R($w^+$) allele; (*a*) red and smooth (wild-type); (*b*) red and roughest (roughest variegation); and (*c*) cream- or cherry-colored and roughest facets (variegation for white and roughest). Schultz (1941a) noted that the R($w^+$) allele, $w^{258\text{-}21}$, of Demerec shows similar relationships; in this case the rough eye variegation of the split gene was followed simultaneously with white variegation. The significant feature in both of these studies was the finding that the breakpoint of the rearrangement occurred to the right of the roughest and in the latter case of the split gene, and therefore to the right of white as well. Representing the point of rearrangement to heterochromatin by a period these rearrangements may be symbolically represented as follows: R($w^+$ $rst^+$.) and R($w^+$ $spl^+$.). The effect exerted by heterochromatin on the newly adjoining euchromatic region was thus seen to spread from the point of new arrangement always effecting first the gene closest to that point (Fig. 2).

The concept of the spreading effect may be applied to the data of Raffel and Muller (1940), concerning the three X chromosome inversions, $sc^4$, $sc^{L8}$, and $sc^{81}$ (Fig. 3). It was found that the left ends of these inversions showed significant and consistent differences from one another with respect to causing a reduction in number of specific bristles and hairs, irrespective of which right end had been combined with them; while the differences exerted by the three right ends, in the presence of

a given left end, were either not significant or were not consistent with respect to any pattern of bristle loss. Bristle reduction was least in the case of the left end of $sc^4$ and greatest in the case of $sc^{L8}$. Two of the sets of specific bristles involved were, as was pointed out, those effected by the achaete (*ac*) gene. This suggests that variegation of $ac^+$ was occurring to different degrees in these three arrangements. The remaining bristle losses by which these inversions could be consistently differentiated were those usually considered to belong to the group affected by the scute gene. Since these inversions had a break in sensibly the same position just to the right of 1B3-4 (Fig. 4) it was concluded that the differences might have to be accounted for by assuming further divisibility of the scute gene, or of genes lying to the right of it. A serious complication however to the analysis of the phenotypes of these inversions is the associated slight hairy-wing effects (noted for $sc^4$ by Alikhanian, 1938; and for $sc^{81}$ by Crew and Lamy, 1940). Sutton (1943b) made the important discovery that achaete probably represents a separate locus to the left of that of Hairy-wing. Thus the spreading effect in the case of these inversions should cause a superposition of a bristle and hair loss pattern due to variegation of the achaete and scute genes, and an extra bristle and hair pattern due to variegation of the Hairy-wing gene lying between them. It should be noted that even were the changes in actions of the scute gene identical and of the stable type in each of the inversions (as discussed in Section IV.2), the differences can still be interpreted in terms of superposition of achaete and Hairy-wing effects, since extra bristles of the scute as well as the achaete type are known to be effected by the Hairy-wing mutant (Dubinin and Sidorov, 1933).

Gersh and Ephrussi (1946) studied the influence of three white mutations, which were known to be deficiencies for 1, 5, and 13 bands lying immediately to the left of, but not including, the white gene, on the variegation of $w^+$ in $w^{m4}$ (Fig. 1), $w^{258\text{-}18}$ (Fig. 2), and $w^{m5}$ (the latter being an X-4 translocation very similar in its breakpoints to $w^{258\text{-}18}$). In the case of the latter two R($w^+$) types significant reductions in viability occurred when either was opposite a chromosome bearing one of the two longer deficiencies as compared to each opposite a normal chromosome. This result can be interpreted on the basis that variegation of genes lying to the left of white was resulting in recessive lethal or semi-lethal effects. It was observed that the R($w^+$) / *Df*, *w* survivors had darker eyes than their R($w^+$) / *w* sisters, a consistent result if the extreme white-variegated types in the former case are inviable. In $w^{m4}$, which gave no appreciable reduction in viability, variegation if it is spreading at all probably proceeds in the direction of genes which lie to the right of the

white locus and which therefore would be "covered" by their normal alleles in the deficient chromosome.

It is noteworthy that the spreading effect demands a concept of a chromosome linearly differentiated into units with specific developmental effects, as in the classical theory of the gene (*cf.* Goldschmidt, 1946). It may be expected that it will serve as a means of identifying effects of new genes. Variegation of the normal allele of the gene, lethal (1) 7, has already led to the discovery that this "lethal" gene is probably an eye color mutant (Schultz, cited in Bridges and Brehme, 1944).

### 5. *Modifiers of Variegation*

The V-type position effects have been found to be extremely sensitive to a variety of modifying factors. Fortunately some of these factors modify the variegation process as such rather than specific variegated-types and thus constitute useful tools for its study. Thus, Gowen and Gay (1933a, 1934) have shown that variegation of the white gene (in the case of three different R($w^+$) types) is suppressed in the presence of an extra Y chromosome, that is, in the XXY female or in the XYY male. Noujdin (1938, 1946a) reported that the addition of heterochromatin of the X, or of the fourth chromosome, or the addition of either arm of the Y chromosome is effective in suppressing variegation (of the yellow gene), although to a less marked extent in each case than the addition of an entire Y chromosome. Schultz (cited by Bridges and Brehme, 1944) found that three Notch variegated-types of Demerec ($N^{264\text{-}6}$, $N^{264\text{-}9}$, $N^{264\text{-}10}$) normally lethal or in one case rarely viable in the male survived in the presence of an extra Y chromosome and no longer show Notch-variegation; curiously such males were sterile.

Schultz (1936) found that the absence of a Y chromosome in the male, the XO condition, caused a marked enhancement of variegation; *i.e.*, an increase in the proportion of mutant tissues; and later (Morgan *et al.*, 1941) noted that a deficiency for heterochromatin of chromosome 2R was almost equally effective in enhancing variegation. Noujdin (1936) found that variegation of the yellow gene in $sc^8$ is increased from less than 1% (per cent of flies having yellow spots) in the XY male to 99% in the XO male.

Gowen and Gay (1933b, 1934) have shown that high temperature leads to a suppression of variegation and low temperature to an enhancement of the process. It was found however that the Y chromosome effect dominated the temperature effect, in the sense that a white-mottled type which showed extreme white variegation at 18°C., was wild type at this temperature if an extra Y chromosome were present. Chen (1948) found that cold treatment (16-17°C.) applied to various stages of the larval

and pupal development of the R($w^+$) strains, $w^{258\text{-}18}$ and $w^{m5}$, was effective in decreasing the amount of eye pigment only when applied in the early pupal period. It is possible however that a more effective period for temperature modification of variegated types exists in the egg stage (see discussion by Schultz, 1941a; and Noujdin, 1945).

Schultz (1941a) has noted several cases in which a rearrangement between the euchromatic and heterochromatic regions acted as a modifier of variegation of another such rearrangement in the same nucleus. There is then the possibility that a V-type rearrangement may act, *per se*, as a modifier of the variegation process, thereby further complicating the analysis of V-type position effects. Although structural heterozygosity is not a necessary condition for the production of the V-type position effect, it becomes important to know whether the structural state can act as a modifier of variegation. An experimental study of this point was made by Gersh and Ephrussi (1946) in the experiments already referred to (Section II.4), in which the influence of deficiencies near the white gene on the phenotype of R($w^+$) heterozygotes was measured. It was found that the same deficiency appeared to modify the phenotype of different R($w^+$) types in different directions. Compared to the control R($w^+$) / $w$ sisters, the eye color averaged darker in the case of $w^{m5}$ / *Df*, $w$ and $w^{258\text{-}18}$ / *Df*, $w$ and lighter in the case of $w^{m4}$ / *Df*, $w$, with either the 5-band or 13-band deficiencies; in the case of the 1-band deficiency, $w^{258\text{-}45}$, R ($w^+$) / *Df*, $w$ was lighter in color than R($w^+$) / $w$ for the three R($w^+$) types studied. When allowance is made, however, for a differential loss of the more extreme white-variegated flies on the basis that variegation of lethal genes was occurring (as discussed in Section II.4), the results can be taken to indicate that the deficiencies resulted in an enhancement of variegation. It is possible that these deficiencies were associated with a change in action of the white gene more extreme than that in the chromosome bearing the mutant white allele and, therefore, that the deficiencies themselves were not influencing the variegation process. Although Gersh and Ephrussi showed that this was not evident from tests comparing *Df*, $w$ with $w$ opposite the apricot allele, $w^a$, the reviewer has found (unpublished) that the deficiency, $w^{258\text{-}45}$, gave a lighter eye color when opposite the eosin allele ($w^e$) than did $w$ (*cf.*, results similar to the latter case with known white deficiencies—Mohr, 1919).

In individuals homozygous for a rearrangement having a V-type position effect, it would be anticipated that less mutant tissue would be present than in individuals which are heterozygous for the rearrangement and which have an extreme mutant allele of the gene in question in the normal chromosome; that is, in the former case, the chance that

an individual cell would have both normal alleles of that gene impaired in function would depend on two separate events, one of which is sufficient for its detection in the heterozygote. Such a result would be expected quite apart from Ephrussi and Sutton's (1944) consideration that the homozygote should be less extreme than the heterozygote on grounds of greater stress on the gene in the latter case imposed by pairing difficulties. There is little reason to believe that the above relation holds in Drosophila, at least for the genes which normally lie in euchromatin; it may, however, apply in Oenothera (Section V). Demerec and Slizynska (1937) report that the R($w^+$) homozygote had lighter eyes than R($w^+$) / $w$, in the case of $w^{258\text{-}18}$; and Schultz (1941a) has remarked that "the homozygotes always show more variegation than the heterozygote." Possibly, somatic pairing in the case of the homozygous rearrangement brings about a much closer association of heterochromatin and the affected pair of alleles, than is possible in the case of the heterozygote, where pairing between the heterochromatic regions in the normal and rearranged chromosome is impaired. Noujdin (1935, 1944) has interpreted results of studies of variegation of the yellow and achaete genes associated with the $sc^8$ inversion in terms of maternal and paternal effects. In this work, the state of structural heterozygosity or homozygosity is assumed to modify not only the degree of mosaicism within the individual but to impose semipermanent changes on the chromosomes transmitted by them. Noujdin (1946b), however, reported that these remarkable properties had disappeared after some generations in some lines of scute-8, which suggests that modifier genes may have played an important role in the earlier experiments. Subjective errors may also have been high since only the number of flies having spots (yellow or achaete) was recorded.

The existence of a diversity of modifier genes (exclusive of Y chromosome effects) which enhance or suppress the variegated phenotype has been inferred from the fact that light and dark lines of mottled-eye types may be readily sorted out by selection (*e.g.*, Demerec and Slizynska, 1937); in other cases (Gowen and Gay, 1934) selection for such lines was ineffective indicating that permanent changes within the affected gene are not at work in the former cases. Schultz (Morgan *et al.*, 1937) reported an autosomal modifier having "a dominant maternal effect for the suppression of variegation." Demerec and Slizynska (1937) found a spontaneous, inherited change, which caused the background in an R($w^+$) line, $w^{258\text{-}18}$, to appear white instead of the usual cream or cherry color.

Such a diversity of modifying factors as that outlined above may well have obscured significant relationships within, and clearly compli-

cate the analysis of, the V-type position effects. In this respect, it should be noted that special precautions, such as those taken in the experiments of Raffel and Muller and those of Gersh and Ephrussi cited above, are needed to minimize the influence of such modifiers in comparative studies of the V-type rearrangements.

### 6. *Reversals of the V-Type Position Effects*

One experimental approach to the study of the V-type position effect has consisted in the induction of further changes within given mottled types by x-ray treatment. One study of this kind was Panshin's (1938) analysis of the R($w^+$) type, $w^{m11}$, (Fig. 2) involving an X-4 translocation in which the white locus was transferred to heterochromatin of the short arm of the fourth chromosome. A total of eight induced "complete reversals" of the variegated phenotype was analyzed cytologically. Seven rearrangements which had transferred the white gene to a euchromatic position, and one case transferring it to the distal heterochromatin of the Y chromosome, were effective in restoring the normal action of the gene. A similar result was obtained in an analysis of thirteen partial reversions of $w^{m11}$; in these cases, however, rearrangements which replaced the heterochromatin of the right arm of chromosome four with euchromatin were effective in reducing the amount of variegation of the white gene in the other arm. On the other hand new derivatives of $w^{m11}$ which were detected as extreme white-variegated types were found to be caused by the presence of additional heterochromatin near the white gene. Griffen and Stone (1940) subjected the translocation, white-mottled-5 (Fig. 2), to further x-ray treatment. Unfortunately, only the results obtained from an analysis of the partial or complete reversions of the white-mottled phenotype were recorded. These results were in agreement with those of Panshin in indicating that such reversions are due to a transfer of the white gene to a new euchromatic position. It is likely that the partial reversions obtained by Panshin and these workers are the result of carrying over some of the heterochromatin from the original attachment to the fourth chromosome to the euchromatic region. As Kaufmann (1942) has pointed out it is also possible that such partial reversions reflect an influence of interstitial heterchromatin on the normal allele of the white gene. If such be the case it is likely that such regions have a very feeble capacity of inducing variegation and such cases would likely have been overlooked in experiments in which the original mottled type is detected. The cytological properties of seventeen x-ray induced partial or complete reversals of roughest variegation associated with the $rst^3$ inversion (Fig. 1) were studied by Kaufmann (1942). In

these cases, transfer of the $rst^+$ gene to a new euchromatic region was the general rule.

Studies of induced changes in variegated types are complicated by the possibility that the x-ray treatment has caused the production of more or less non-specific suppressors and enhancers of variegation. Schultz (Morgan *et al.*, 1937) has pointed out that some of the new x-ray induced derivatives of an R($bw^+$) type obtained by Dubinin (1936) may have been of this nature, especially those derivatives in which new rearrangements had occurred at some distance from the brown locus and had acted as modifiers of the dominant brown phenotype.

The frequency with which reversions of variegated types arise is expected to be high if the sole requirement is the removal of the gene from its new heterochromatic association to any non-heterochromatic region. In the case of $w^{m5}$, such conditions are probably essentially similar to those required in the case of position effects of the $ci^+$ gene (Section III.2), (*cf.*, the position or $ci^+$ in the normal fourth chromosome, with the position of $w^+$ in $w^{258\text{-}18}$, which is similar to $w^{m5}$ except that in the former $w^+$ probably immediately adjoins heterochromatin of the fourth chromosome, Fig. 2). Griffen and Stone (1940) cite data of Mickey indicating 0.3% "true reversals" of $w^{m5}$ among 7,015 tested gametes at a dosage of 4,000 r units. The frequency with which a weakened dominance of $ci^+$ is obtained following x-ray treatment at this dosage may be taken as being within the range of 0.3% (Muller, 1930) and 1% (Stern *et al.*, 1946), intermediate values having been found by Khwostova and Gavrilova (1935, 1938). Kaufmann (1942) estimated a frequency of 0.4% reversals of roughest variegation in $rst^3$ (4,000 r units). The high frequency with which reversions of variegated types is obtained provides additional indirect evidence that such rearrangements still carry the normal allele of the gene in question as expected on the position effect hypothesis. The question of whether the mutability of such wild-type alleles is increased when they are adjacent to heterochromatin is discussed in the next section.

### 7. *The Question of Germinal Stability*

Muller (1930), Gowen and Gay (1934) and Demerec (1941a) have noted that R($w^+$) types maintain the inheritance of the somatically unstable phenotype with no evidence that stable reversions or stable mutant changes in the rearranged $w^+$ allele occur spontaneously. In the progeny of males carrying the $sc^8$ inversion (Fig. 3) a high spontaneous rate of lethal changes resembling simultaneous mutation of the yellow and achaete genes occurs. Sidorov (1940, 1941a) found that the rate of these changes was 0.019% among 250,366 tested gametes and demon-

strated that these types arise as the result of crossing over between the X and Y chromosomes. It was established that such crossovers involved the distal heterochromatin contained in the scute-8 inversion and the short arm of the Y chromosome (pairing in reverse order). The lethal yellow-achaete changes represented X chromosomes in which the tip of the chromosome had been replaced by the tip of the short arm of the Y chromosome. On this scheme a complementary crossover type carrying the tip of the X chromosome attached to the proximal part of the short arm of the Y chromosome is expected, and was detected by a special test. The addition of such a Y chromosome and the complementary, deficient X chromosome restores the normal balance, and was found to give a variegated phenotype identical with that of the original $sc^8$ chromosome and a normal Y chromosome. Crew and Lamy (1940) also obtained a crossover chromosome having the tip of $sc^{81}$ (Fig. 3) attached to the Y chromosome in the above manner and found that such an attachment did not alter the variegation of genes in that region. Such exceptions, then, have served to prove the rule; namely, that variegation is a process which is confined to the somatic cells of an organism. The same rule seems to apply in the case of position effects in Oenothera (Section V).

The high mutability of the y and ac genes in the $sc^8$ inversion when x-rayed (Patterson, 1932a; Sidorov, 1936; Belgovsky, 1939; and Muller, 1940) is in part due to the deficient types of crossovers with the Y chromosome, occurring spontaneously, as pointed out by Sidorov (1941a). Among the non-lethal changes in these loci which were induced, it was proposed, see Muller (*loc. cit.*), that minute rearrangements of these genes had occurred. Possibly, rearrangements involving the heterochromatin adjoining these genes in the inverted chromosome contributed to some of these by producing extreme variegation of these genes.

### 8. *Cytological Aspects of Variegation*

There is general agreement (Schultz and Caspersson, 1939; Prokofyeva-Belgovskaya, 1939; Cole and Sutton, 1941) that the euchromatic regions of the salivary gland chromosomes tend to lose their capacity to stain as sharply banded regions when they are brought into close proximity to heterochromatin as in the V-type rearrangements, just as the distinction between euchromatin and heterochromatin in the normal chromosomes is not clearly defined (see divisions 19 and 20 in Fig. 4). Prokofyeva-Belgovskaya suggested that this represents a weakening of the conjugational properties of the chromonemata of the euchromatic regions as a result of proximity to the loosely organized heterochromatin. An analysis of this effect was made by Caspersson and Schultz (1938)

and by Cole and Sutton (1941), who have measured the relative amounts of ultraviolet absorbing substances in specific euchromatic bands brought next to heterochromatin. In these studies the R($w^+$) type. $w^{258\text{-}21}$ (Fig. 2), was investigated. The breakpoint in the X chromosome in this translocation was found to lie between the bands 3E5 and 3E6

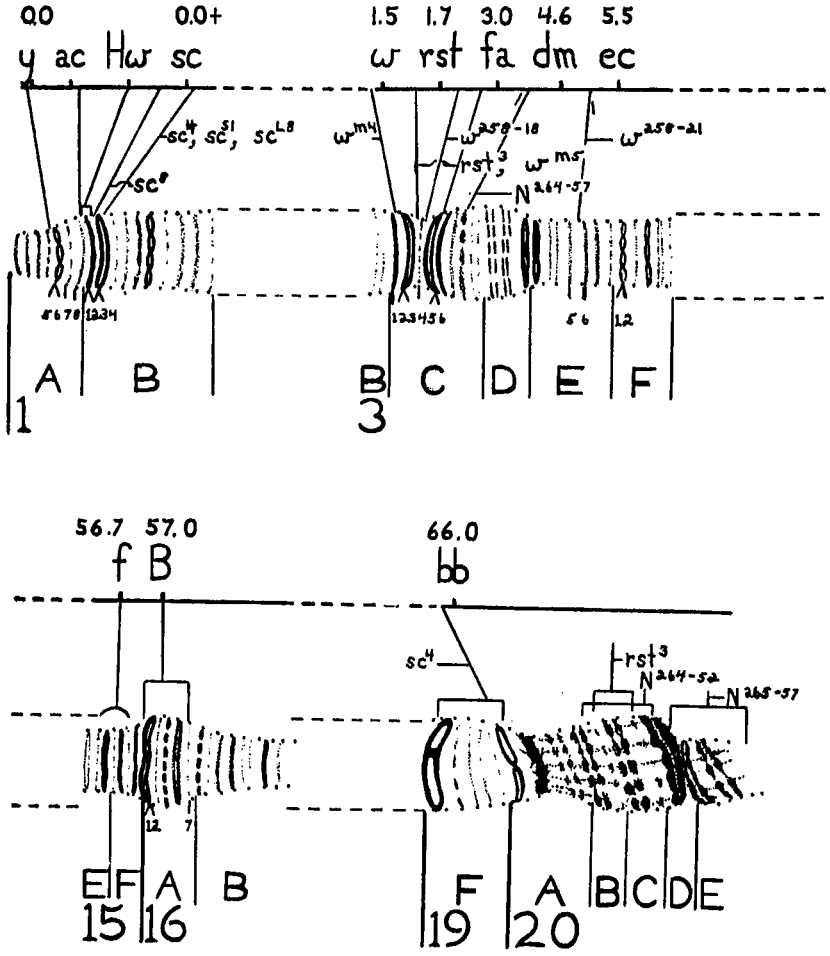


FIG. 4

Genetic and cytological correspondences in four regions of the X chromosome which have been widely studied for position effects. Details and references are contained in the work of Bridges and Brehme (1944) and in the text. The drawing of the salivary gland chromosome is based on Bridges' (1938) revised map of the X-chromosome.

(Fig. 4) and Demerec (1942) found that variegation of the genes, *w*, *rst*, *fa* and *dm*, but not *ec* occurs. From measurements of the double band 3F1-2, Schultz and Caspersson concluded that an increase in the nucleic acid content of this band appeared when it was adjacent to heterochromatin in the rearranged chromosome as compared to the amount found in the same band of a normal chromosome present in the same nucleus. On the other hand, Cole and Sutton (1941), using a similar type of technique, were unable to demonstrate consistent differences in the density of absorbing substances in the bands 3E1-2 and 3C2-3; the latter two bands being associated with the white gene which was known to show variegation in this rearrangement. The later discovery (Demerec *et al.*, 1942) that the locus of echinus is associated with the 3F1-2 band, suggests, since no variegation for this gene was reported, that the differences in intensity of absorption of this band have no direct bearing on the variegation process as it is detected genetically by means of changes in gene activity. Prokofyeva-Belgovskaya (1939) observed that the chromonemata or interband regions which ordinarily do not take the Feulgen stain in the euchromatic regions begin to stain when such regions are brought next to heterochromatin, in a manner similar to that of the interband regions of normal heterochromatin (see section 20 in Fig. 4). More recently (1945, 1947), she has reported that the degree to which a euchromatic region resembles heterochromatin in the case of the V-type rearrangements depends on whether the rearrangement is homozygous or heterozygous and on the previous history of the chromosome, that is, whether or not it was derived from a male parent or the female parent. It was claimed that complete confirmation of the results of Noujdin (1944) on the paternal and maternal effects and the state of structural heterozygosity or homozygosity on variegated types was obtained (see Section II.5).

## III. The Behavior of Genes Located in or near Heterochromatin

### 1. *Variegation of the Light Gene*

A rearrangement which involves euchromatin and heterochromatin may in some cases be associated with a change in action of a gene lying in or near the heterochromatic region concerned. In one case, that of the light gene ($lt^+$) in the left arm of the second chromosome it is clear that the change may be of the variegated type (Schultz and Dobzhansky, 1934). Although several rearrangements have been recorded as having a light variegated effect and a correlated break in the heterochromatin of the left arm of the second chromosome (Schultz, 1936), little is known about the necessary conditions which call forth the posi-

tion effect in this case. It has however been noted that variegation of the light gene in such cases is suppressed in the XO male and enhanced, that is, more mutant tissue is present, in individuals with extra Y chromosomes (Schultz, *loc. cit.*). The latter then constitutes the inverse of the relationship found for variegation of genes located in the wholly euchromatic regions. These results suggest that the V-type position effects may be approached in two ways which in a sense complement each other; on the one hand, there is the antagonistic effect of heterochromatin on the action of genes normally lying remote from it, and at the same time, there is an indication that some genes depend for their normal functioning on proximity to heterochromatin. The cubitus interruptus gene (*ci*) which lies in the neighborhood of, if not within, the proximal heterochromatin of the fourth chromosome appears to represent another example of the latter phenomenon as discussed below; here, the phenotype of the mutant *ci* is highly variable and confined largely to a single wing vein, so that the detection of a variegated-phenotype in the case of position effects of this gene is scarcely possible.

### 2. *The Dubinin Effect*

Position effect of the cubitus interruptus gene, sometimes referred to as the "Dubinin effect," was first discovered by Dubinin and Sidorov (1934a, 1934b) who found that ten among nineteen translocations involving the fourth chromosome showed a weakened dominance of $ci^+$ when they were tested against a chromosome bearing the recessive, *ci* mutant. Such R($ci^+$) types, or "position alleles" (following the terminology of Stern and Heidenthal, 1944) were remarkable in that the hemizygotes, which were obtained for four of the original ten cases, and the homozygotes viable in two cases possessed normal venation in contrast to the gaps which appeared in the fourth vein of R(+) / *ci* and *ci* / *ci* genotypes. Moreover, eight combinations made up by combining two different R($ci^+$) types also gave normal venation. Two examples of R ($ci^+$) types, $w^{258\text{-}18}$ and $w^{258\text{-}21}$, are shown in Fig. 2. The validity of the position effect hypothesis was best attested to by Khwostova's (1939) extensive study by the salivary gland chromosome method of 196 x-ray induced *ci* changes. Of these 193 were associated with chromosomal rearrangements and for the most part only rearrangements of the euchromatic-heterochromatic type gave the Dubinin effect; in all cases a break had occurred in the proximal heterochromatic region of chromosome four. These results had already been foreshadowed by the extensive genetic analysis of R($ci^+$) types by Panshin (1935). Only two types of exceptions to the eu-heterochromatic relation were observed: (*a*) rearrangements involving the fourth chromosome and the distal as opposed

to the proximal heterochromatic regions of the X or Y chromosomes, gave the Dubinin effect (*cf.*, Panshin's (1938) finding of a similar anomaly in the case of reversals of white variegation—Section II.6) and (*b*) those involving the heterochromatic regions or the adjoining euchromatic regions of the autosomes gave the effect only when such regions were removed through inversion from the centromere region. Dubinin *et al.*, (1935) showed that the break in the proximal heterochromatin of the fourth chromosome need not be identical in different R(*ci*$^+$) types, it being to the left of the bent gene in one case and to the right of this locus in another. It was evident that the position effect spread through the locus of bent but no detectable effect on the bent gene was observed. Stern *et al.* (1946) also found that the breakpoint in two R(*ci*$^+$) types had occurred to the left of the cubitus interruptus gene.

Khwostova's plot of the observed breaks in the euchromatic region of 193 R(*ci*$^+$) types revealed no evidence for excessive grouping of breaks in any given region, and such grouping as was observed appears to be of the same type expected if some regions show a somewhat higher breakage frequency per unit salivary gland chromosome length than others (Bauer *et al.*, 1938). Her results did however reveal a possible significant departure from randomness in that breaks in the euchromatic regions immediately adjoining the centromere regions of the other autosomes were not found. It may be assumed that any rearrangement involving the removal of the *ci*$^+$ gene from heterochromatin of the fourth chromosome and its transfer to any euchromatic region, not immediately adjoining the proximal heterochromatin, will show a weakened dominance of *ci*$^+$.

If the position effect of the *ci* gene were due solely to structural heterozygosity then it would follow that R(+) / *ci* and R(*ci*) / + would be identical in phenotype when R is the same rearrangement in each case. Although such a comparison has not yet been technically feasible, Stern *et al.* (1946) have obtained indirect evidence that it can not play a major role in evoking the Dubinin effect just as it played little or no role in the V-type position effects of the hairy and curled genes (Section II.2). After treating wild type and *ci* males with the same dosage of x-rays and mating separately to *ci* and to wild-type females respectively, it would be anticipated, were R(+) / *ci* and R(*ci*) / + in general identical in phenotype, that individuals with vein interruptions of the *ci* type would arise with equal frequencies in the two experiments, other factors being equal. From the mating of *ci* females and treated wild-type males (4,000 r units), 39 individuals having gaps in the fourth vein were obtained among a total of 4,358 progeny reared at 26°C.; using

the same x-ray dosage and temperature conditions, they found that the reciprocal mating gave no individuals of this type in a comparable number of progeny (4,639). In the latter as well as in the former experiment, several individuals were obtained having a slight *ci* phenotype, consisting in a thinning of the fourth vein. Earlier experiments (Stern and Heidenthal, 1944) had shown that rearrangements involving the *ci* mutant, R(*ci*), detected on the basis that they gave a more extreme *ci* phenotype opposite *ci* than did the *ci* homozygotes, give a slight *ci* phenotype in the heterozygote with a normal allele and that this dominance is enhanced at 18°C. (see also Sidorov, 1941b). Although this result might suggest that the action of the $ci^+$ allele in the normal chromosome had been directly interfered with, it is known that a much more striking dominant *ci* effect can be given by either of two dominant mutations, $ci^W$ (Wallace) and $ci^D$, the latter known to be apparently normal cytologically (Bridges, 1935b).

For an adequate appreciation of the nature of position effect of the *ci* gene it becomes particularly necessary to consider the properties of the mutant alleles of this gene when they lie in their normal position. Such a study has been given by Stern (1943) and Stern and Schaeffer (1943a, 1943b). It was determined that three doses of the *ci* mutant gene (the triplo-IV condition) lead to more nearly normal venation than do two doses (diplo-IV), which in turn were more nearly normal than one dose (either as the haplo-IV condition or in tests opposite a deficiency, Minute-4, for the *ci* gene). The mutant, $ci^W$, on the other hand was nearly normal in one dose and very extreme in venation abnormality in two doses. These and other relationships with the R(+) and R(*ci*) types will be summarized here by the use of series expressing the relative degree of venation disturbance. Thus, when *ci* and $ci^W$ are compared with each other by testing each opposite a chromosome bearing *ci* (or $ci^W$), then the following seriation results with reference to $ci^+$ (where the sign, >, means has more nearly normal venation than):

$$+ > ci > ci^W \qquad (1)$$

If, however, the same set of alleles is tested against a deficiency for the *ci* gene the position of *ci* and $ci^W$ in the series was significantly reversed as follows (where the sign, $\geqq$, means equivalent to or more nearly normal than):

$$+ \geqq ci^W > ci. \qquad (2)$$

The same contradiction expressed in series one and two was later (Stern *et al.*, 1946) found to hold for *ci* and those R(+) alleles which were especially selected because they gave greater vein defects in the het-

erozygote with *ci* than did *ci* homozygotes. The seriation opposite *ci* was, therefore, by definition:

$$+ > ci > \mathrm{R}(+) \tag{3}$$

In tests opposite a deficiency the same three alleles gave the following series:

$$+ \geqq \mathrm{R}(+) > ci \tag{4}$$

In complete confirmation of the above results of Dubinin and Sidorov, the four R(+) types appeared wild-type in the hemizygote. Series 4 also held true when the test chromosome was the same R(+) allele; again three of the four R(+) types gave normal venation in the homozygote; in the exceptional case designated, in Stern's notation, $\mathrm{R}^2(+)$, the homozygote was very similar to the *ci* homozygote. This exception was notable in another respect; namely, it had involved a break in the left arm of the fourth chromosome. In the light of Panshin's (1938) discovery that sixteen rearrangements having breaks in the short arm of the fourth chromosome did not show a Dubinin effect, it is possible that the $\mathrm{R}^2(+)$ allele had in fact an associated mutant allele of *ci* induced by the x-ray treatment at the same time as the rearrangement; *i.e.*, its effect appears to conform to that of the R(*ci*) type, described below.

Finally, similar relationships were obtained with R(*ci*) alleles, selected because they gave the following seriation with respect to *ci* in tests opposite *ci*:

$$+ > ci > \mathrm{R}(ci) \tag{5}$$

More recently (Stern, 1948b) it was reported that R(*ci*) types in general give normal or nearly normal venation in the hemizygote, so that the following series holds in tests opposite a deficiency:

$$+ \geqq \mathrm{R}(ci) > ci \tag{6}$$

It had earlier been shown (Stern and Heidenthal, 1944) that series 6 also holds if the test is made opposite an R(+) type.

Series 1 through 6 summarize some of the complex relationships observed with position alleles of the *ci* gene. Whatever plays a role in causing the shift in position of *ci* and the position allele in series 3 and 4, or 5 and 6, may well be related to the factor or factors responsible for a similar shift observed for *ci* and $ci^W$ as between series 1 and 2. In a sense, the R(+) alleles of series 3, act more like a deficiency for the gene than like either *ci* or $ci^W$; possibly the *ci* phenotype results from an accumulation of one substance relative to another produced in an adjoining region (or locus) such that the partial inactivation of the

whole region, which might occur in R(+), would fail to give the *ci* phenotype (see discussion of other genes having dominant mutant alleles in Section II.1). The R(*ci*) alleles, by comparison with the R(+) alleles more closely resemble the $ci^W$ allele in that (*a*) they are slightly dominant to the normal allele, (*b*) the hemizygote approaches normality, (*c*) and the homozygote is as extreme or more extreme (Stern, 1948b) than the *ci* homozygote. But series 1 and 2 indicate that the transition between a *ci* and $ci^W$ type of change would involve a discontinuity. Such a discontinuity is observed, however, among different R(*ci*) alleles: those which give the least dominance of the *ci* phenotype (closest to *ci*) opposite a normal chromosome bearing $ci^+$ give the greatest amount of vein interruption when opposite an R($ci^+$) allele (Stern and Heidenthal, 1944). Further clues may be obtained when results of tests of the R(*ci*) and R(+) types with $ci^W$ or $ci^D$ alleles are reported.

Some of the observed relationships between the mutant alleles and position alleles of the *ci* gene could be explained (Stern *et al.*, 1946) by assuming that the gene possesses two, more or less independently varying attributes, (*a*) its "combining power," c, for a substrate (S), and (*b*) an "efficiency," e, with which S is converted into a gene product (P). Since the effectiveness of a particular allele in its production of P must be represented by the product, c·e, the hypothesis proved insufficient to account alone for any one of the three discrepancies which are expressed by the above series. In the case of series 3 and 4, many facts could be brought into line if it was assumed that (*a*) the $ci^+$ allele in the rearranged chromosome has a reduced amount of substrate available in its new position, and (*b*) competition between alleles in homologous chromosomes occurs in R(+) / *ci* such that *ci* has a priority, by virtue of its normal position, over the R(+) allele for S. In the R(+) homozygote or hemizygote this competition would no longer be present and the $ci^+$ allele could still lead to normal venation even in the presence of reduced amount of S, because of its relatively high combining power and efficiency in comparison to the *ci* allele. Additional assumptions would have to be made however to fit in the later results expressed by series 5 and 6 in which the *ci* allele in the R(*ci*) hemizygote appears to function in a more nearly normal fashion than when it is in its regular position. Stern (1948a) also obtained evidence which strongly indicated that competition between alleles for a common substrate would have to occur independently of whether or not the alleles are close together as in the structural homozygote, or are far removed, as in one R(+) allele where a minute region of the fourth chromosome carrying the $ci^+$ allele was transferred and inserted to another chromosome.

Stern (1948b) suggested that qualitatively different phenomena may

be involved in some of the R(*ci*) types. In several independent occurrences of these, the rearrangement had a break within a narrow region of the right arm of the second chromosome and at the same time these cases were stated to be exceptional in having a break to the right of the *ci* gene instead of to the left which appears to be the rule for the R(+) alleles and presumably other R(*ci*) alleles. Whether this result indicates that a specific type of association with the *ci* gene has occurred with respect to genes located in this region of the second chromosome, or whether the effect is due to a position effect on genes in that region which enhances or simulates the *ci* phenotype is not clear. Although the position effect interpretation can, in most cases, be considered valid for the known R(+) rearrangements giving the Dubinin-effect, the evidence suggests that the R(*ci*) types represent a complex group of changes not all of which necessarily involve position effects of the *ci* gene itself.

If the Dubinin effect is considered as another example of variegation then the behavior of the R(+) types might be explained by assuming that in the homozygote the addition of a variegation pattern for the *ci* character would tend to give wild-type because the probability that the alleles in both chromosomes would be effected simultaneously would be low; to explain the fact that the hemizygote is wild-type it might be assumed that the deficiency for heterochromatin of the fourth chromosome in such individuals would by analogy with the effect of the loss of the Y chromosome on the variegation of the light gene (Section III.1) cause a suppression of the variegation. Panshin (1936) and Khwostova (1939) have both reported that R(+) alleles are more extreme in the XXY female than in the XX female; the published data indicate that this effect is rather slight and not always consistent. Results of tests in the XO male have not appeared. The variegation hypothesis cannot be regarded as very satisfactory and would not explain the finding that the R(*ci*) hemizygote has normal or nearly normal venation.

## IV. The Stable-Type Position Effects

### 1. *Position Effect without Chromosomal Abberration*

Position effects associated with a stable change in gene action are rare by comparison with the number of examples of V-type position effects. Indeed there are no established cases in which a stable change arising in association with a chromosomal rearrangement has been proven to be due to position effect rather than to intragenic mutation. Proofs of the stable type position effects have been obtained, however, in other types of phenomena: (*a*) The behavior of different dosages of the Bar region as studied with the Bar duplication (Section IV.3) and (*b*) the

apparent allelism exhibited by the closely linked genes, Star (*S*) and asteroid (*ast*). In the latter case (Lewis, 1945), a quite different phenotype is obtained depending on whether the two mutants are in the same chromosome and their normal alleles in the other, that is, *S ast* / + + as compared to the equivalent genotype having the mutants in opposite chromosomes, namely, *S* + / + *ast*. This example of the position effect did not depend on the presence of a chromosomal rearrangement since each of these mutants appeared normal in the salivary gland chromosomes. In the former case the phenotype was equivalent to that of *S* / + in having a slightly smaller than normal rough eye; while in the latter case extreme reduction in the size and roughness of the eye occurred in all individuals. A similar comparison involving another asteroid allele, $ast^4$, also showed a marked difference in phenotype between the equivalent genotypes. Finally a comparison of the two equivalent genotypes, *S ast* / + $ast^4$ and *S* $ast^4$ / + *ast* indicated that the former produces a larger eye than the latter. From females of the type, *S ast* / + +, an asteroid mutant allele was recovered as a crossover on three occasions and possessed the same properties as the original asteroid mutant. A cytogenetic analysis of these mutants showed that they were correlated with a doublet structure (see Fig. 5; other examples: 1B3-4, 19F1-2, in Fig. 4) near the end of the left arm of the second chromosome. It was therefore suggested that these exceedingly closely linked loci might represent an instance of a duplication, now

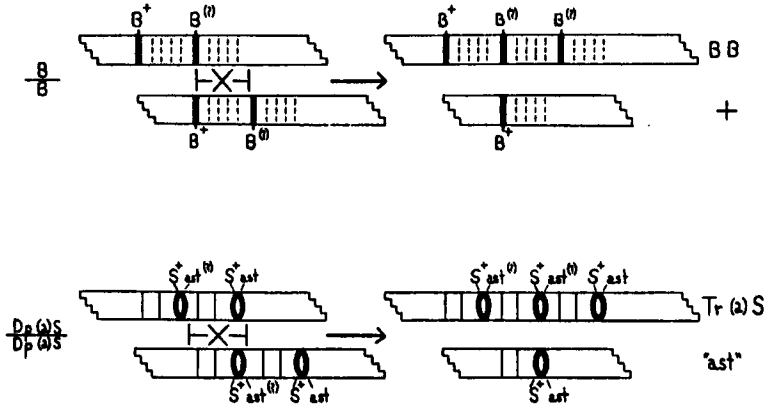


FIG. 5

Diagrammatic representation of the origin of normal chromosome and triplication types from contiguous, direct duplications of the Bar (B) and Star (Dp-S) type. Uncertainty as to the presence of a stable mutation as opposed to position effect of a gene is indicated by (?). See text for description.

established in the species, of a single ancestral gene. Another case of linked genes, namely, bithorax and bithoraxoid, exhibiting a position effect of this sort has been noted (Lewis, 1948). The evidence suggests that the two genes involved in each of these pseudo-allelic series are similar but not identical in function. Two simple models suggest themselves: (*a*) the two genes are competing for the same substrate (S), (*b*) each gene controls a separate step in a reaction series of the following type: S → A → B. Results of studies with the bithorax and bithoraxoid mutants (unpublished) support the latter model. It is suggested that the position effect may in some cases be brought about by the direct dependence of one gene upon the reaction product of its neighbor gene in the same chromosome and that these products are produced in such limited amounts, or diffuse so slowly, that each chromosome behaves more or less independently of its homolog with respect to these reactions. The possibility that duplicate genes may often diverge by a process whereby one comes to control a reaction successive (or antecedent on the Horowitz (1945) hypothesis) to that controlled by the old, receives support from an independent consideration of enzyme specificities. Thus in a reversible reaction series of the type: $A \rightleftharpoons B \rightleftharpoons C$, the enzymes which control these two steps may in many cases have to be structurally very similar to one another if they are to share a common specificity for the intermediate product, B. Such a specificity might only be possible in the case of enzymes produced by genes which were once identical.

Bridges' (1935a) interpretation of the doublet structures in the salivary gland chromosomes as one-band duplications and their widespread occurrence throughout the chromosomes, suggests that there may be many pairs of closely linked, functionally related, genes in this organism. Wherever their functioning is dependent upon close proximity a basis is provided for detecting position effects of the S-type. Rearrangements, especially those associated with stable, as opposed to variegated-type, changes in gene action may in some cases be position effects on genes of the above type.

### 2. *Possible Examples of the S-type Position Effects*

A considerable number of chromosomal rearrangements in Drosophila have been found to be associated with stable as opposed to variegated changes in gene action. As a rule, such rearrangements involve the wholly euchromatic regions of the chromosomes and the associated stable change exhibits quite different properties from that of variegation of genes accompanying the eu-heterochromatic rearrangements. Demerec (1941a, 1941b) and his coworkers have described many such cases in-

volving stable changes at such loci as *w, rst, N,* and *ct.* The locus of the affected gene was usually adjacent or only a few bands removed from the point of rearrangement. It was noted, therefore, that the sensitive region of the gene change was much narrower in these cases than in the V-type position effects. Significantly, there was no evidence of the spreading effect (Section II.4); that is, an extreme change usually resembling inactivation of the gene occurs when the gene is one or several bands removed from the point of rearrangement, while the intervening loci remain unaffected.

A common example of the above phenomenon is the high frequency with which chromosomal rearrangements of x-ray origin are found to be associated with lethal changes near the point of breakage. Although these have often been assumed to be the result of position effect, Lea and Catcheside (1945) found that the existing data relating x-ray dosage to the frequency of production of sex-linked recessive lethals do not require such an assumption, it being sufficient to assume that in a certain proportion of rearrangements a lethal gene mutation is induced at the same time (estimated roughly to be about three in eight breaks). They estimated that the maximum proportion of lethals of the position effect type, which could be reconciled with existing data (Timoféeff-Ressovksy, 1939) relating dosage to sex-linked lethal frequency, would not be likely to exceed 17%. It should be noted that some lethals of the position effect type are expected in the case of X-chromosome rearrangements having extreme variegation effects, but even the proportion of these may be low; examples are several of the Notch-variegated types which act as lethals in the XY male, but which survive in the XYY male (Section II.5), indicating that no permanent change within the gene has occurred. Kaufmann (1947) found that when the frequency of rearrangements following x-ray treatment at 4,000 r units was increased by pretreatment with infrared radiation, the proportion of lethals associated with rearrangements diminished with respect to the proportion in the control, which was treated at the same dosage but without the infrared pretreatment. This was the result expected on the basis that few position effect lethals occur, but the results were not statistically significant. On the basis of extensive cytological analyses of x-ray induced sex-linked lethals and their rate of production over a wide range of doses, Dubinin *et al.* (1941) have also concluded that lethals arise largely as the result of a primary effect of the x-rays.

It is likely that the frequency with which lethal or "visible" gene changes occur in Drosophila in association with chromosomal rearrangements is too high to be accounted for on the basis of chance coincidence of two independent processes or "hits." Demerec and Fano (1941) have

concluded that a "single-event" process may account for the production of most of the short (less than 15-band) deficiencies of x-ray origin. It is therefore reasonable to assume that mutation may sometimes be induced simultaneously with chromosomal breakage in genes several bands removed from, as well as those adjacent to, the breakpoint; just as Hoover's (1938) cytological analysis of a selected group of eighteen breakage points revealed the presence of small deficiencies at these points in five cases. An example derived from the V-type rearrangements is the inversion, $N^{264\text{-}57}$ (Fig. 1), in which the stable change at the roughest locus, several bands removed from the point of rearrangement, may be viewed as an intragenic mutation, if not loss, of this gene (Demerec, 1941a).

There is some possibility that certain genes, such as, scute, cut, Star, and asteroid, exhibit a stable-type position effect when rearrangements occur directly adjacent to the gene. In the case of the latter pair of closely lined genes, there was some reason to suppose that breaks did not actually occur between them but that breaks to the right of the asteroid locus when induced in a normal chromosome, were effective in producing an inactivation of both genes; or curiously, if *ast* was x-rayed, reverse (non-lethal) mutations of asteroid were found to be associated also with a break immediately to the right of these genes (Lewis, 1945). Breaks and new reunions which occur immediately to the right of the band 1B3-4 (Fig. 4) are associated with a whole series of scute changes (see discussion by Muller, 1941); Sutton (1943b) found additional cases of such changes, however, in which the break near scute had occurred to the left of this doublet, and even in some cases to the left of 1B1-2. Unfortunately, it is very difficult to obtain an unselected sample of breakage points close to specific loci. Sutton, however, in the above work obtained several rearrangements having breaks just to the left of 1B3-4 or 1B1-2 detected as yellow changes, which did not have associated effects on the scute gene; similarly, breaks just to the left of and one to the right of the Star-asteroid loci did not have associated changes in these genes, in a total of three cases analyzed (Lewis, *loc. cit.*).

One means of testing the position effect hypothesis in the case of stable changes accompanying rearrangements is to try to induce reversals of that change on the assumption that the occurrence of a new rearrangement of the gene in question might restore its normal action. Raffel and Muller (1940) did not obtain reversals of the scute-4 phenotype, associated with the $sc^4$ inversion (Fig. 3), among "approximately 50,000" male offspring from a mating of attached-X females and treated (3,000 r units) $sc^4$ males. Goldat (1936) obtained no reverse mutations of the scute-7 change, associated with a rearrangement of the wholly

euchromatic type (having a break just to the right of scute, as in $sc^4$), among 46,842 daughters from a mating of scute females (which were also yellow and achaete-3) and treated (4,000 r units) $sc^7$ males, where the occurrence of any reversals associated with recessive lethals could have been detected. Reversals of the dominant phenotypic effect of certain changes associated with chromosomal aberrations, such as, Dichaete and Lyra (Dubovsky and Kelstein, 1938), or Glazed (Griffen and Stone, 1939) may not be cogent, since here it is likely that partial or complete inactivation of the gene concerned would also lead to such a reversal; that is, such cases may still have been lethal or abnormal in the homozygous condition. An apparent reversal of the Punch phenotype, a dominant non-variegated eye color change, associated with a rearrangement, was lethal when homozygous and abnormal in combination with Punch, as shown by Oliver (1941).

Another somewhat more probable example of the S-type position effect than those discussed above, is the finding that x-ray induced Bar-like (small-eye) phenotypes (or induced reversals of such phenotypes in the case of Bar itself) are often accompanied by a rearrangement having a break very near the 16 A1-2 bands (Figs. 4 and 5). In these cases, however, the situation appears much more complex and difficult to analyze as discussed in Section IV.3.

A special application of the problem of the origin of the stable change in action associated with rearrangements is that of deficiency-bearing chromosomes, in which such a change may occur in a gene close to, but not actually included in, the deficient segment. Such a deficiency then becomes totally unsuitable as a means of identifying the cytological location of that gene with any precision. Examples of such cases were found by Sutton (1943b) in the case of the yellow and scute genes. These cases are puzzling if one assumes that the locus of the gene has to be within the deficient segment, for one would then have to conclude, as Goldschmidt (1944) has done in this case, that within a whole region near the tip of the X chromosome the 'locus' of yellow could be practically anywhere. The accurate location of genes in the chromosomes clearly demands much more refined methods, namely, the use of only those rearrangements which do *not* have associated stable changes in the action of the gene or genes in question. It was by the use of such rearrangements that Sutton (1943b) was able to obtain an unequivocal placing of the yellow and achaete genes in the region from 1A5 to 1A8, inclusive (See Fig. 4).

### 3. *Position Effect and Intrachromosomal Duplications*

As the result of the independent cytological studies of Bridges (1936) and Muller *et al.* (1936), it is now known that the original case of position effect found by Sturtevant (1925) depends on the way in which four doses of a given region of the X chromosome are distributed with respect to homologous chromosomes (rather than two, as originally supposed). Thus, the Bar "mutation," *B*, was found to be a duplication in tandem, or contiguous, direct order for the bands of division 16A of the salivary gland X-chromosome, and the unequal crossover product (see Fig. 5) known as double-Bar (*BB*) was shown by Bridges to be a serial triplication for this region. It will be recalled in this case that the position effect was in evidence by the fact that *B* / *B* females had significantly larger eyes than those of the equivalent genotype, *B B* / +; more recently, Chevais (1943) has shown that this difference is maintained when individuals of these two genotypes are fed on the so-called $B^+$ substance, which results in significant increases in the number of eye facets in each case. The fact that attached-X females of these two genotypes likewise can be distinguished by the difference in their facet number suggests that the Y chromosome has little influence, if any, on this position effect (L. V. Morgan, 1931). Although the position effect hypothesis may be considered established in this case, the nature of the change which brings about the Bar phenotype is by no means clear. Rapoport (1936, 1940a) synthesized higher derivatives of the Bar duplication and obtained a five-fold repetition of the Bar region, called quadruple-Bar, $B^q$ or *BBBB*, from homozygous *BB* females. A comparison of *BB* / *BB* with *BBBB* / + females indicated that the latter probably had significantly smaller eyes than the former. Eye size diminished with increasing doses of the Bar region until only a few facets remained in the *BBBB* male.

Results of studies with an enhancer of Bar, symbol, *E-B*, have been reported by Bonnier *et al.* (1943) and Bonnier *et al.* (1947). The locus of this change appeared to be either near the right end of the right section of the duplication or somewhat beyond. The mutant acted as a recessive lethal change. A position effect comparison was possible in the case of females of the following genotypes: *B* + / + *E-B* and *B E-B* / ++; the former, which were similar to *B* / *B* in phenotype, had consistently larger eyes than the latter.

It may be seen from a diagram of the Bar duplication (Fig. 5) that a new rearrangement of genes has been accomplished at the point where the end of the first or left section of 16 A joins the beginning of the second section. Griffen (1941, and Bridges and Brehme, 1944) noted from a

cytogenetic analysis of a rearrangement which had separated the two sections at approximately this same point, the Bar-Stone translocation, that the extreme dominant Bar effect was associated only with the second or right section. A Bar effect was induced, however, in what had constituted the original left section, by further x-ray treatment and in this case a new association of the 16 A1-2 doublet contained therein had occurred. Slight dominance of Bar-like effects which did not depend upon the presence of a duplication for the Bar region have also been found in two other cases to have involved a break very close to 16 A1-2; *i.e.*, just to the left in the case of $B^{263\text{-}48}$, analyzed by Sutton (1943a); and, according to Bridges (Morgan *et al.*, 1936), between 16 A1 and 2 in the case of "Baroid," studied by Dobzhansky (1932, 1936). These results then point to the conclusion that there are at least two separate components of the Bar phenotype to be considered, (*a*) *a stable change in the action of a gene or genes lying next to the point of rearrangement, possibly within the 16 A1-2 doublet at that point, and* (*b*) *an influence somehow exerted by the close juxtaposition of two sets of identical loci.*

Dubinin and Volotov (1936, 1940) and Sutton (*loc. cit.*) found that the change which produces the reversal of the dominant Bar phenotype (or that of double-Bar) can also arise without cytologically detectable change in the duplication (or triplication) or elsewhere. A genetic analysis of one such case by Dubinin and Volotov (1940) revealed that it resembled *B* / *B* when opposite *B*, even though the homozygous females and males had normal eyes. From females homozygous for this apparent reversal of Bar, Bar-like sons were derived. These proved to be associated with crossing over (in the forked-Beadex region) and proved to be triplications for the Bar region, as in *BB*; their frequency was 0.04% (among 16,364 sons) which corresponds to that obtained by Sturtevant (1928) for what are now recognized as triplication types. Their method of analysis did not permit a decision between the possibility that this change was a dominant suppressor mutant, linked, perhaps closely, to the Bar duplication, or a change near the point of rearrangement in the Bar duplication. Rapoport (1941) found the analysis of x-ray induced modifications of the Bar phenotype, was complicated by the induction of suppressor and enhancer mutants at other loci.

In all of the above studies in which reversals of the dominant Bar phenotypes appeared, it was found as expected on the basis of a known Bar deficiency that loss of the 16A region does not give a Bar effect nor does it enhance or suppress Bar, that is *B* / *Df-B* resembled *B* / + (Bridges, 1917). Again the problem as to the nature of a gene change which can produce such results arises (Section II.1); if the bands 16A1-2 are considered as representing two closely linked genes with similar

effects (Section IV.1) the problem becomes a very complex one, indeed, in the Bar case.

Demerec and Hoover (1939) found that the Hairy-wing mutant was associated with a tandem duplication of the bands 1B1-2, at the left tip of the X chromosome (Fig. 4), and suggested that the associated phenotype was the result of a position effect as in the case of the Bar duplication. Spontaneous reversions of Hairy-wing (or triplications) following unequal crossing over are not expected with an appreciable frequency in this case, assuming the duplication to be of the direct type (*i.e.*, ABAB), because of the excessively low crossing over in this region of the X chromosome; Rapoport (1940b) reported none among over 33,000 offspring from homozygous Hw females. Rapoport obtained two reversals of the dominant Hw effect following x-ray treatment (3,000 r units) among 9,005 tested gametes; these proved to be lethal and were evidently deficiencies. Schultz (Morgan *et al.*, 1941) found that the dominant, Confluens mutant was associated with a tandem duplication of a section involving the Notch locus, and assumed a position effect was at work.

A cytogenetic analysis of the Star-duplication (Fig. 5), which includes the Star and asteroid genes and which arose from homozygous females showed that the mutant allele in the right section of the duplication had remained unchanged whereas the asteroid gene in the left section behaved as though it had reverted to wild type (Lewis, 1941, 1945). Extensive tests of the possibilities that the left section still carried a mutant asteroid allele were negative, however. In a whole series of position effect comparisons which were made possible by introducing various combinations of alleles of the Star and asteroid genes into the duplication there was no evidence for the existence of a position effect extending from the loci in one section of the duplication to those in the other. A comparison of the homozygous duplication versus a triplication of this region opposite a normal chromosome gave identical (but practically wild-type) phenotypes in each case. A comparison of the homozygous triplication (having slight venation and facet abnormalities) with the equivalent genotype carrying a quintuplication for this region in one chromosome and a normal complement of genes in the other chromosome likewise failed to show evidence of a position effect.

## V. Position Effect in Oenothera blandina

An important advance in position effect studies came with the discovery and proof of position effect in *Oenothera blandina* (Catcheside, 1939, 1947a). A remarkably close parallel exists between this case and the V-type position effects of Drosophila. The position effect was

detected in a rearrangement, symbol, A, involving the 3.4 and 11.12 chromosomes. Variegation was observed for the dominant alleles, $P^r$ (red sepal-color) or $P^s$ (light red sepals, recessive to $P^r$ but incompletely dominant to the lower alleles, $P$ and $p$, causing green sepal-color) of the $P$ gene, and for the $S$ ($S$, causing yellow petal-color; $s$, sulfur-colored petals) gene located in arm 3. It was found that R($P^s$) / $P^r$ had deep red sepal-color in the flower buds as with $P^r$ / $P^r$ and $P^r$ / $P^s$ genotypes, while R($P^r$) / $P^s$ had variegated sepals containing deep red patches and green areas intermingled. Since the homozygous $P^s$ plant has light red sepal-color (with narrow green stripes) the direction of change in $P^r$ is evidently towards one of the lower alleles, $P$ or $p$. Variegation also appeared in plants of the type, R($P^s$) / $P^s$, these having variegated sepals with light red tissue like that of $P^s$ / $P^s$ and green tissue like that of $P^s$ / $p$, therefore, no change of $P^s$ towards $P^r$ was in evidence.

It was shown that $P^r$, or $P^s$, loses its unstable action when extracted from the rearrangement by crossing over; and a return of these alleles to the rearranged chromosome resulted in a complete restoration of the variegated behavior. A total of 58 transfers of these alleles in and out of the A interchange was detected. In a single homozygous R($P^r$) plant obtained, sepal-color variegation was much less extreme than in R($P^r$) / $P^s$, and was such as "would be expected if two $P^r$ variegations were superposed" (Catcheside, 1947a) (contrast with Drosophila examples, Section II.5).

Variegation of the $S$ allele could be detected when the normal chromosome carried the $s$ allele. Thus plants of the type R($S$) / $s$ had yellow- and sulfur-colored petals, while introductions of $s$ into the interchange to give R($s$) / $S$ plants led to uniformly yellow-colored petals. Variegation of at least the $S$ gene was found to be influenced widely by environmental conditions.

Rarely, variegation with respect to the $P$ locus led to large patches of green tissue comprising whole branches of the plant; within some cases of this kind an occasional bud was variegated or even wholly red. However, progeny derived from green buds of the wholly green branches were again variegated. Evidence of a "spreading effect" (Section II.4) was obtained in a case in which genetic analysis had indicated the presence of a duplication for the $P$ and $S$ genes in the interchange chromosome (Catcheside, 1947b). In such a chromosome it was found that the variegation process affected only the $P$ and $S$ loci lying nearer to the point of rearrangement, thus ruling out the possibility that the variegated pattern represented losses of the distal portion of the translocated arm by an unstable breakage mechanism. Although cytological proof was lacking, it seemed likely that the break in the A interchange had oc-

curred in the proximal arm of chromosome three, presumably within heterochromatin (see Marquardt, 1937).

## VI. Interpretations of the Position Effect Phenomenon

There exist two general hypotheses regarding the mechanism of the position effect. The first involves the assumption that it is primarily related to immediate or early gene products; *e.g.*, Sturtevant (1925), Offerman (1935), and Stern and co-workers (see Section III.2); on this basis the gene itself is assumed to remain unaltered. Ephrussi and Sutton (1944) have called this the kinetic hypothesis. The latter workers and Muller (1941, 1947) have argued for a structural hypothesis, which is based on the assumption that the gene itself is altered, but in a way which is readily reversible and which may be analogous to structural, as opposed to chemical, changes in large protein molecules. Either of these hypotheses has two fundamental aspects of the problem to consider: (*a*) intrachromosomal position effects due, *e.g.*, to the association of a gene with heterochromatin as in the V-type position effects, whereby the effect can be manifested in the total absence of a homologous chromosome, as in the XO male, and (*b*) the possibility that position effects may occur in which an influence extends from one chromosome to its homolog. In the latter consideration, advocates of the structural hypothesis assume that the forces of somatic pairing operating in an individual heterozygous for a chromosomal rearrangement exert a stress, imposed by pairing difficulties, on the genes located near the points of rearrangement; while on a kinetic hypothesis it would be assumed that such forces acting in a structural homozygote would facilitate interactions (*e.g.*, competition) between gene products produced in one chromosome and those produced in the homolog by bringing homologous loci into close proximity, and that these interactions break down in the structural heterozygote. This consideration, however, can be disposed of as a primary factor in the production of the known and established types of position effect in either Drosophila or Oenothera. That is, the position effect has been demonstrated in several cases to be manifested as between two identical structural heterozygotes, *e.g.*, in R(+) / *h* and R(*h*) / +, where R was the same rearrangement in each case and the phenotypes corresponded to a variegated-hairy effect and to wild-type, respectively (Section II.2), or similar examples in Oenothera, (Section V), where somatic pairing itself would be more or less unlikely as Catcheside (1947a) has discussed.

There is the distinct possibility that "interhomolog" position effect is sometimes superposed on the intrachromosomal type. Gersh and Ephrussi (1944) performed experiments to test the possibility of a modi-

fying influence of structural heterozygosity on the manifestation of a variegated phenotype. As already discussed (Section II.5) the evidence is not yet conclusive that a specific modification of the position effect was induced in this case. The possibility of such an action remains an attractive one, since it lends itself very well to experimental test. It has been noted that some evidence suggests that, in the case of the V-type position effects, the modifying influence may be to cause the structural homozygote or hemizygote to be *more* extreme than that predicted for the structural heterozygote (Section II.5); evidently structural homozygosity of the heterochromatic regions might be a more potent factor in modifying the adjoining euchromatic gene, than any stresses exerted on the latter by pairing forces in the structural heterozygote.

In the case of the intrachromosomal position effect, which must be regarded as the fundamental one, a decision between the kinetic and structural hypothesis is far more difficult. In the case of the V-type position effects, the spreading of a structural disturbance initiated within the heterochromatic region by stresses from non-homologous pairing forces (*e.g.*, Ephrussi and Sutton, *loc. cit.*), or the spreading, *along the chromosome*, of some substance possibly nucleic acid, produced in the heterochromatic region and interfering with gene reproduction (Schultz, 1941b) represent alternative but sufficiently non-specific processes to accord with the finding that variegation effects are exerted upon genes without regard to their specific functions (Section II.1 and II.4). In the case of the S-type position effects, far more specific associations of genes appear to be required. In such cases the concept of localized interactions between gene products may constitute a better working hypothesis than the structural one (Section IV.1).

The V-type position effects have established a division of the genetic material into two major components, which happen to correspond, perhaps not accidentally, with euchromatin and heterochromatin, as seen in the salivary gland chromosomes. This type of position effect may well be very widespread in other animals and in plants, especially judging by the example of position effect in Oenothera. On the other hand, the evidence from the Drosophila and Oenothera studies indicates that a great number of conditions may have to be met before it is possible to detect the phenomenon. It is possible to conceive of many situations in which the V-type position effect would be present but not detectable; *e.g.*, in the case of Drosophila itself, its detection is often made impossible in the presence of extra Y chromosomes, presumably meaning an excess of heterochromatin with respect to euchromatin.

The S-type position effects suggest that the chromosomes are organized not wholly at random with respect to the specific roles which the

genes are to play in development; but rather that there occur, perhaps in most organisms, instances of adjacent genes which are related in function probably as the result of an origin by duplications of a single gene (Section IV.1). When such genes are dependent for their normal functioning on close proximity, the possibility of detecting a position effect arises; otherwise proximity probably reflects only a more or less recent origin of such genes. One of the practical consequences of such a relation is that the separate genes which exhibit a positional dependence may act like multiple alleles of a single gene, in a phenotypic sense. Examination of apparent multiple allelic series, or closely linked genes, from this standpoint are under way in many cases and may reveal the existence of the S-type position effect in other organisms (*e.g.*, Dunn and Caspari, 1945; and Laughnan, 1949).

There have been isolated reports of the occurrence of very slight phenotypic changes, which might be slight V- or S-type position effects, associated with rearrangements (Brink, 1932; Jones, 1944; Roberts, 1942, in the case of maize; and see, *e.g.*, Goldschmidt *et al.*, 1939, in the case of Drosophila). Here, however, it would be all but impossible to exclude the possibility that differences within modifying genes were responsible for the apparent position effect.

## VII. Summary

Position effects in Drosophila can be divided into two rather distinct categories: (*a*) the variegated- or V-type, associated with a mosaic phenotype, and (*b*) the stable- or S-type, in which a phenotypic change occurs which is similar to, if not identical with, that of a stable intragenic mutation. The V-type appear to be detectable whenever a gene normally lying in euchromatin is brought next to heterochromatin, provided certain other conditions, not fully understood, obtain. They are subject to an almost bewildering number and variety of modifying factors. It is clear that the "variegation of a gene" is not initiated by structural heterozygosity but is an intrachromosomal phenomenon somehow brought about by an influence of heterochromatin, and possibly only specific portions of such material, on genes abnormally set next to it. The fact that the change in gene action does not occur in every somatic cell, the fact that no germinal instability has been detected, and the ready reversibility of the change all suggest that the gene itself has not been altered in any permanent way; finally, it has been possible to prove this in several instances. Variegation of a gene lying in or near the heterochromatic region may occur when it is abnormally set next to euchromatin; whether position effects of the cubitus interruptus gene are of this type is not clear.

The S-type position effects do not require a chromosomal rearrangement as a necessary basis for their detection; when one is present, it often involves the wholly euchromatic regions of the chromosomes. The S-type, when associated with x-ray induced rearrangements, is open to confusion with the phenomenon whereby x-rays appear to cause chromosome breakage and intragenic mutation in a gene close by as the result of a single "hit." The valid cases of the S-type position effect probably depend for their existence on the presence of specific functional relationships between neighboring genes; rearrangements which separate such genes, the artificial production of such genes as the result of an intrachromosomal duplication of the Bar-type, and intragenic mutation within one of such genes, provide means whereby a position effect of the S-type may be detected. The position effect will be present however only in those cases in which proximity of such genes is a requirement for their normal functioning.

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