V-TYPE POSITION EFFECTS OF A GENE IN DROSOPHILA VIRILIS NORMALLY LOCATED IN HETEROCHROMATIN [1]

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THE general problem of the relationship between stable chromosome rearrangements and somatic variegation has been reviewed recently by LEWIS (1950) and HANNAH (1951). Although many cases in Drosophila are known in which the variegation is caused by placing heterochromatin adjacent to or near genes normally located in euchromatic regions, the only extensive study that has been made of the factors responsible for variegation of characters determined by genes normally located in heterochromatin is that of KHVOSTOVA (1939) on the cubitus interruptus (*ci*) gene in *Drosophila melanogaster*. In the work being reported, 32 different chromosome rearrangements in *Drosophila virilis* are described, each of which produces variegation of the eye pigmentation when heterozygous with the recessive allele of the gene peach whose locus is normally in heterochromatin. An abstract of a portion of this work has been published (BAKER 1952b).

This gene is the closest known locus to the centromere of chromosome 5, being 203 crossover units from the tip of this apparently telocentric chromosome. Two recessive alleles of the gene are known, pe^{lap} and *pe*. The latter, when homozygous, produces a lighter eye color than the former and is the allele used in practically all the crosses to be discussed. All the rearrangements responsible for the peach-mottled eyes were induced by X-rays in sperm of wild-type males of the Pasadena strain. The majority of these R(+) rearrangements (pe^{m5} through pe^{m53}) came from treatments of 5000 r.

RESULTS

Prior to describing the rearrangements which evoke the variegation, a few general remarks which apply to all cases of mottling are in order. In every case tested thus far, the mottling is recessive, i.e., flies of the genotype R(+)/+ are wild type, and R(+)/*pe* have variegated eyes. The eye in most of the mottles appears to be a mixture of wild-type and peach pigmentation, although in some cases (frequently in pe^{m1}) the darkly pigmented areas are definitely darker than wild type. The pattern of variegation is not wholly consistent in the two eyes of a single individual or among flies having the same rearrangement, and therefore it is impossible to differentiate the various mottles by their phenotypic expression (see fig. 1). In general, the pattern may be characterized by

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stating that the variegated eyes are composed of a few patches of mutant or wild-type tissue in the complementary background rather than a salt-and-pepper distribution of the pigmentation. There is little evidence of diffusion of pigments from one ommatidium to an adjacent one, each ommatidium being either mutant or wild type. The pattern of variegation usually extends across

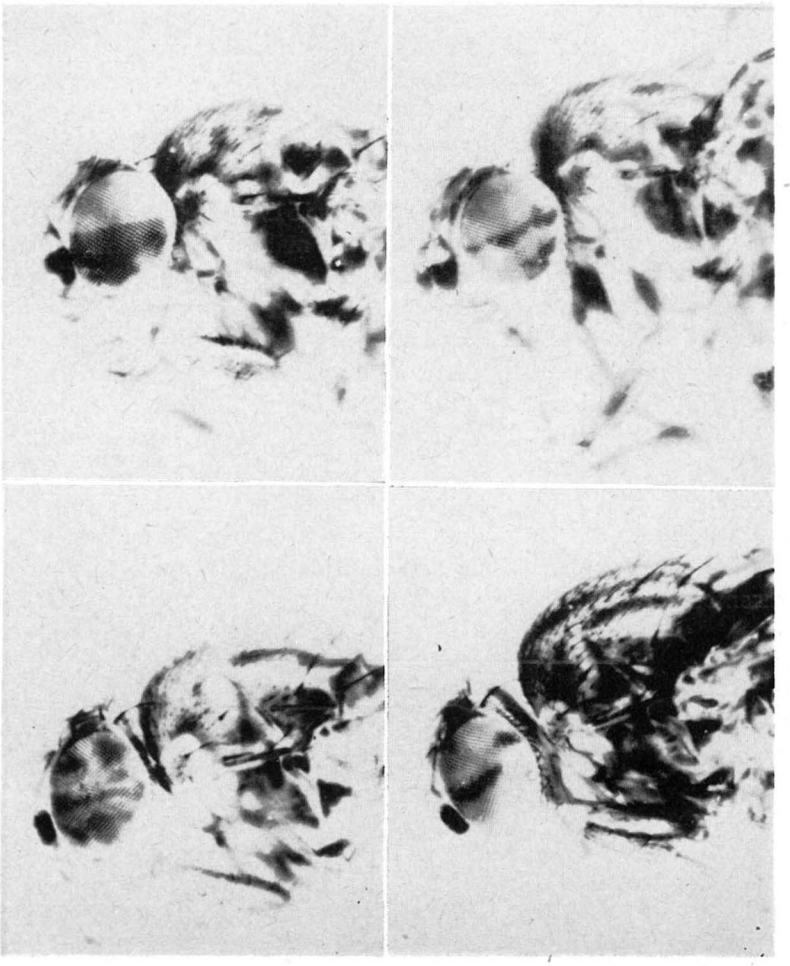


FIGURE 1.—Variations in the phenotypic expression of $T(Y;5)pe^{m1}$. The dark areas in the eye have wild-type pigmentation whereas the light areas are peach.

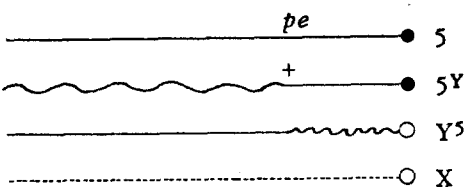
the eye in the anterior-posterior direction; no clearly dorsal-ventral patterns have ever been observed. A line separating the dorsal and ventral halves of the eye appears to influence the pigment distribution since two of the most common patterns are ones in which the dorsal half of the eye is one color and the ventral half the other color (fig. 1) and ones in which all the eye is peach (or wild type) with the exception of a line of facets separating the dorsal and ventral halves (fig. 1). In the majority of mottles the variegation is not so striking as

in the case of pe^{m1}. In many cases most of the eye is wild type with only one or a few small patches of peach pigmentation.

Peach-mottled 1

This first case of variegation involving the *pe* locus was discovered in an F_1 male from the progeny of irradiated wild-type males which were crossed to *b; t; cd; pe* (markers on chromosomes 2, 3, 4 and 5, respectively) females. When this male was testcrossed to the marked females, there was no evidence of linkage between chromosomes 2, 3 or 4, but it could be inferred that a translocation had taken place between the Y and chromosome 5 since all the females resulting from the testcross were phenotypically peach and most of the males were mottled. A similar testcross made at a later time gave the following results in a generation composed of 4103 flies: 51.7 percent peach females, 39.7 percent mottled males, and 8.6 percent peach males. Although these data indicate the presence of a Y; 5 translocation, a careful study of the salivary gland chromosomes and metaphase configurations found in larval ganglion cells failed to reveal any chromosome abnormalities. Therefore, both chromosomes have been broken at approximately the same distance from their centromeres.

In order to elucidate the structure of the translocation, mottled males were crossed to wild-type females and the resulting F_1 males were crossed to peach females. The results of this cross and the subsequent tests made on the F_2 offspring are shown in table 1. If it is assumed that chromosome 5 has been broken distal to the *pe* locus and that the 5^Y chromosome is responsible for the variegation, then the results shown in this table become understandable. Thus the chromosome configuration of a mottled male is assumed to be as follows:



The F_1 wild-type males would have the same configuration, but the wild-type allele of peach would be on the normal chromosome 5. Alternate segregation in the F_1 male translocation heterozygote would produce mottled males and wild-type females when mated to peach females, while adjacent-2 segregation would give wild-type males and peach females if flies which are hyperploid or hypoploid for the proximal region of 5 are viable. The validity of this interpretation is established by the offspring of crosses (2) and (3), which show that the F_2 wild-type males carry the 5^Y chromosome which produces the variegation. They further indicate that the F_2 peach females carry the Y^5 chromosome which has a deletion for the *pe* locus. It should be noted that, genetically or cytologically, it is impossible to differentiate between the Y^5 chromosome and a normal chromosome 5 carrying the *pe* allele. Thus it is impossible to distinguish between mottled males of the type $5/Y^5/5^Y$ and those of the constitution $5/5/5^Y$. After it was found that $5/5/5^Y$ males carried the translocated

chromosome causing variegation and were fully fertile, stocks of this translocation were maintained by mating the mottled male offspring (from the cross peach females to F_2 wild-type males) to peach females. Thus all the mottled stocks are of a known constitution, namely, $5/5/5^Y$.

The F_2 peach males remain unaccounted for on this explanation. It is obvious from cross (4) that they contain the 5^Y chromosome, but phenotypically the mottling has gone to completion (i.e., all the eye is mutant tissue) since in every case they produced mottled male offspring when crossed to peach females.

It is interesting to note that, when the peach males which are genetically like the mottled males are added to the latter class, adjacent-2 segregation and alternate segregation are equally frequent in this translocation heterozygote. In addition, it has been found that 3-1 segregation is almost entirely absent.

TABLE 1

Crosses verifying the constitution of pe^{ml}.*

Cross	Mottled ♂♂ × wild-type ♀♀ F_1 are all wild type						
	Percentage of offspring in the various classes						
	Wild ♂♂	Mot ♂♂	pe ♂♂	Wild ♀♀	pe ♀♀	Total No.
(1) F_1 wild ♂♂ × pe ♀♀	25.1	17.9	6.3	25.6	25.0	9363
(2) F_2 wild ♂♂ × pe ♀♀	26.3	20.3	2.2	26.8	24.3	3561
(3) F_2 pe ♀♀ × pe ♂♂			~50		~50	Not counted
(4) F_2 pe ♂ × pe ♀♀†		36.7	11.4		51.9	3233

*The author is grateful to Mrs. Chloris Dow, who gathered the data given in this table

†Data from 27 individual males.

This type of segregation should produce mottled females with a chromosome configuration identical to that shown above with the exception that they would have two X chromosomes. Many thousands of flies have been examined from crosses of mottled males to peach females and only one mottled female has been found. When crossed to peach males, she produced 281 offspring consisting of 23.5 percent mottled males, 23.8 percent peach males, 22.0 percent mottled females, and 30.6 percent peach females. Cytological preparations of the ganglion cells from some of the F_1 male larvae from this cross revealed the presence of an extra chromosome. This would be expected, since all the F_1 mottled males would have a normal Y chromosome in addition to the Y involved in the translocation. The presence of this extra Y produces no striking effect on the amount of mutant tissue present in the variegated eyes.

Peach-mottled 15

The variegation in this case is also the result of a translocation, which cannot be observed in salivary chromosomes, between chromosome 5 and the Y. When mottled males were crossed to peach females, the following phenotypes

were observed with the indicated frequencies among the 559 progeny: mottled males, 30.6 percent; peach females, 32.4 percent; mottled females, 19.9 percent; and peach males, 17.2 percent. The relatively low frequency of mottled females and peach males is an indication of the presence of a Y; 5 translocation. Since the F_1 peach males were all completely sterile, it would be suspected that they arose from aneuploid gametes which lack all or most of the Y chromosome. Thus it would be anticipated that the F_1 mottled females arose from nondisjunction of the X chromosome and the translocated chromosome carrying 5 attached to most of the Y. The fact that these females are mottled would indicate that chromosome 5 is broken proximal to the *pe* locus. In order to verify the location of the break in chromosome 5, mottled males were crossed individually to wild-type females, and the resulting F_1 males were crossed to peach females. Since no peach flies were observed among the progeny of the latter cross, it is clear that the break is proximal to the *pe* locus. One interesting fact became apparent upon examination of the ganglion chromosomes in the F_1 male larvae from the above cross. Some of the parental males produced male offspring, all of which had only nine rod-shaped chromosomes, eight normal and one long. Other parental males produced both males of this chromosome constitution and ones with one long, one short and eight normal rod-shaped chromosomes. The males bearing only nine rod chromosomes proved to be fertile. It is thus evident that the translocated chromosome, bearing the tip of the Y attached to the centromere of 5, is not necessary for viability or fertility in the male. From a consideration of pe^{m1} and pe^{m15}, it is apparent that neither the tip of the Y nor the region near its centromere is essential for male fertility.

CYTOLOGICAL ANALYSIS OF REARRANGEMENTS

It is not practicable to give a complete description of the phenotypic expression and genetic tests which have been accomplished on each of the rearrangements. It is sufficient to say that all the mottles have been tested genetically to determine whether any translocations have taken place between chromosomes 2, 3, 4, 5 or 6 which were not observed cytologically. Such tests are necessary since, as will be seen from the following descriptions, one break in chromosome 5 invariably is in the basal heterochromatin and, if this is accompanied by a break in another basal heterochromatic region, the rearrangement may not be observable in salivary gland preparations. In every case the genetic tests verified the cytological picture although additional breakage points (in heterochromatin) were indicated in certain rearrangements.

Cytological preparations of the larval ganglion and salivary gland chromosomes were made using the squash technique. The salivary glands were placed in 1 *N* HCl for 30 seconds before staining with aceto-orcein. Permanent slides were made of all rearrangements using a freezing-drying technique made available to the author by DR. A. D. CONGER (BAKER 1952a). The regions of the salivary chromosomes denoted in the following descriptions are taken from the chromosome map of this species prepared by GRIFFEN (PATTERSON *et al.*

1940). In denoting the manner in which the broken ends are joined in the rearrangement, the free end of the chromosome is shown at the left and the centromere end at the right. The chromosomes are separated from each other by semicolons, the rejoined breaks within a chromosome are separated by commas.

Peach-mottled 1 Baker 47i24 $T(Y;5)pe^{m1}$. No rearrangement is visible in either salivary gland or ganglion chromosomes.

Peach-mottled 2 Baker 51b26 $T(3;5)pe^{m2}$. Two breaks in 5: between 5A7a and b, and between 5H1d and 5H2a. One break in 3 between 3C6a and b. Rearrangement: 3C6a-b to 5H1d-5H2a; 5A7a-b to 5H1d-5H2a, 5A7a-b to 3C6a-b.

Peach-mottled 3 Baker 51c2 $T(4;5)pe^{m3}$. No rearrangement is visible in the salivary gland chromosomes.

Peach-mottled 4 Baker 51c30 $T(3;5)pe^{m4}$. One break in 5 between 5H2d and centromere. One break in 3 between 3B2e and h. Rearrangement: 3B2e-h to 5H2d; 5H2d to 3B2e-h.

Peach-mottled 5 Baker 52a25 $T(3;5)pe^{m5}$. Three breaks in 5: between 5G6c and 5G7a, between 5H1b and 5H2a, and presumably between 5H2d and centromere. One break in 3, presumably in 3H1. (The proximal break in 5 cannot be observed cytologically, and the only cytological evidence of the break in 3 is the high frequency of asynapsis in the basal end of this chromosome. However the genetic evidence clearly indicates a translocation between 3 and 5.) Rearrangement: 3H1 to 5H2d; 5G6c-5G7a to 5H1b-5H2a, 5G6c-5G7a to 5H1b-5H2a, 5H2d to 3H1.

Peach-mottled 11 Baker 52a28 $T(2;5)pe^{m11}$. One break in 5 between 5H2d and centromere. One break in 2 between 2A4b and e. Rearrangement: 2A4b-e to 5H2d; 5H2d to 2A4b-e.

Peach-mottled 12 Baker 52a29 $T(2;5)pe^{m12}$. One break in 5 between 5H2d and centromere. One break in 2 between 2B8a and d. Rearrangement: 2B8a-d to 5H2d; 5H2d to 2B8a-d.

Peach-mottled 15 Baker 52a29 $T(Y;5)pe^{m15}$. No rearrangement is visible in salivary gland chromosomes, but one long and one short chromosome are evident in ganglion cells.

Peach-mottled 16 Baker 52a29 $T(4;5)pe^{m16}$. One break in 5 between 5H2d and centromere. One break in 4 between 4D1b and e. Rearrangement: 4D1b-e to 5H2d; 5H2d to 4D1b-e.

Peach-mottled 18 Baker 52a30 $T(4;5)pe^{m18}$. Two breaks in 5: between 5B2f and g, and between 5H2d and centromere. One break in 4 between 4F1c and d. Rearrangement: 4F1c-d to 5H2d; 5B2f-g to 5H2d, 5B2f-g to 4F1c-d.

Peach-mottled 19 Baker 52a30 $T(2;3;5)pe^{m19}$. Two breaks in 5: between 5G4a and b, and between 5H2d and centromere. One break in 3 in proximal end of region 3A1. One break in 2 in 2H2 region. Rearrangement: 5G4a-b to 5H2d; 2H2 to 5G4a-b, 5H2d to 3A1; 3A1 to 2H2.

Peach-mottled 20 Baker 52b2 $T(2;5)pe^{m20}$. Three breaks in 5: between 5C5f and g, between 5H1d and 5H2a, and between 5H2d and centromere. One

break in 2 between 2C1a and b. Rearrangement: 2C1a-b to 5H1d-5H2a, 5C5f-g to 5H2d; 5C5f-g to 5H1d-5H2a, 5H2d to 2C1a-b.

Peach-mottled 22 Baker 52f12 *T(4;5)pe*^{m22}. One break in 5 between 5H2d and centromere. One break in 4 between 4A6a and c. Rearrangement: 4A6a-c to 5H2d; 5H2d to 4A6a-c.

Peach-mottled 24 Baker 52f12 *T(1;5)pe*^{m24}. One break in 5 between 5H2d and centromere. One break in 1 between 1E5b and e. Rearrangement: 1E5b-e to 5H2d; 5H2d to 1E5b-e.

Peach-mottled 25 Baker 52f14 *T(4;5)pe*^{m25}. Two breaks in 5: between 5B4d and f, and between 5H2d and centromere. One break in 4 between 4A3a and c. Rearrangement: 5B4d-f to 5H2d; 4A3a-c to 5H2d, 5B4d-f to 4A3a-c.

Peach-mottled 29 Baker 52f16 *T(3;5)pe*^{m29}. One break in 5 probably between 5H2d and centromere. One break in 3 between 3C5a and c. Rearrangement: 3C5a-c to 5H2d; 5H2d to 3C5a-c.

Peach-mottled 31 Baker 52f16 *T(4;5)pe*^{m31}. One break in 5 between 5H2d and centromere. One break in 4 between 4D1a and e. Rearrangement: 4D1a-e to 5H2d; 5H2d to 4D1a-e.

Peach-mottled 32 Baker 52f16 *T(1;2;5)pe*^{m32}. Two breaks in 5: between 5G5c and d, and between 5H2d and centromere. One break in 1 between 1D2a and d. One break in 2 between 2E8a and c. Rearrangement: 1D2a-d to 5H2d; 2E8a-c to 5G5c-d, 5H2d to 1D2a-d; 5G5c-d to 2E8a-c.

Peach-mottled 33 Baker 52f16 *T(1;4;5)pe*^{m33}. One break in 5 between 5H2d and centromere. Two breaks in 1: between 1F2c and 1F3a, and between 1G3h and 1G4a. Two breaks in 4: between 4D5a and d, and between 4G2b and e. Rearrangement: 4D5a-d to 1G3h-1G4a, 1F2c-1F3a to 5H2d; 5H2d to 4D5a-d, 4G2b-e to 1G3h-1G4a; 1F2c-1F3a to 4G2b-e.

Peach-mottled 34 Baker 52f16 *T(4;5)pe*^{m34}. One break in 5 between 5H2d and centromere. Two breaks in 4: between 4E1g and h, and between 4H3b and d. Rearrangement: 5H2d to 4H3b-d, 4E1g-h to 5H2d; 4E1g-h to 4H3b-d.

Peach-mottled 35 Baker 52f16 *T(2;5)pe*^{m35}. One break in 5 between 5H2d and centromere. One break in 2 between 2D4b and d. Rearrangement: 2D4b-d to 5H2d; 5H2d to 2D4b-d.

Peach-mottled 36 Baker 52f16 *T(2;5)pe*^{m36}. One break in 5 between 5H2d and centromere. One break in 2 betwen 2A2c and e. Rearrangement: 2A2c-e to 5H2d; 5H2d to 2A2c-e.

Peach-mottled 37 Baker 52f16 *T(2;5)pe*^{m37}. Two breaks in 5: between 5B5b and 5C1a, and between 5H2d and centromere. One break in 2 between 2B2g and 2B3e. Rearrangement: 2B2g-2B3e to 5H2d; 5B5b-5C1a to 5H2d, 5B5b-5C1a to 2B2g-2B3e.

Peach-mottled 38 Baker 52f16 *T(1;5)pe*^{m38}. One break in 5 between 5H2d and centromere. One break in 1 between 1D8f and 1D9b. Rearrangement: 1D8f-1D9b to 5H2d; 5H2d to 1D8f-1D9b.

Peach-mottled 39 Baker 52f18 *T(2;5)pe*^{m39}. One break in 5 between 5H2d and centromere. One break in 2 between 2D2g and 2D3a. Rearrangement: 2D2g-2D3a to 5H2d; 5H2d to 2D2g-2D3a.

Peach-mottled 41 Baker 52f18 $T(4;5)pe^{m41}$. Two breaks in 5: between 5E6e and 5E7a, and between 5H2d and centromere. One break in 4 between 4D2i and 4D3c. Rearrangement: 5E6e-5E7a to 5H2d; 4D2i-4D3c to 5E6e-5E7a, 5H2d to 4D2i-4D3c.

Peach-mottled 42 Baker 52f18 $T(4;5)pe^{m42}$. One break in 5 between 5H2d and centromere. One break in 4 between 4B3c and 4B4a. Rearrangement: 4B3c-4B4a to 5H2d; 5H2d to 4B3c-4B4a.

Peach-mottled 45 Baker 52f19 $T(1;5)pe^{m45}$. One break in 5 between 5H2d and centromere. One break in 1 between 1C1a and c. Rearrangement: 1C1a-c to 5H2d; 5H2d to 1C1a-c.

Peach-mottled 46 Baker 52f19 $T(1;5)$ pe^{m46}. One break in 5 between 5H2d and centromere. One break in 1 between 1C7b and d. Rearrangement: 1C7b-d to 5H2d; 5H2d to 1C7b-d.

Peach-mottled 50 Baker 52f25 $T(1;5)pe^{m50}$. Two breaks in 5: between 5A3f and i, and between 5H2d and centromere. One break in 1 between 1E2c and d. Rearrangement: 5A3f-i to 5H2d; 1E2c-d to 5H2d, 5A3f-i to 1E2c-d.

Peach-mottled 51 Baker 52f25 $T(3;5)pe^{m51}$. One break in 5 between 5H2d and centromere. One break in 3 between 3B2c and d. Rearrangement: 3B2c-d to 5H2d; 5H2d to 3B2c-d.

Peach-mottled 53 Baker 52f26 $T(3;5)pe^{m53}$. One break in 5 between 5H2d and centromere. One break in 3 between 3B2a and b. Rearrangement: 3B2a-b to 5H2d; 5H2d to 3B2a-b.

Evidence that pe is normally in heterochromatin

The evidence that the *pe* locus is contained within the last four bands of heterochromatin at the base of chromosome 5 (see plate I A) comes from an analysis of three rearrangements, pe^{m1}, pe^{m3} and pe^{m4}. The conclusive evidence in the case of pe^{m1} that the break in chromosome 5 is distal to the locus has been stated. However, a searching analysis of the salivary chromosomes has failed to reveal any abnormality. Similarly, pe^{m3} shows no visible rearrangement in the salivary chromosomes although genetic evidence indicates the presence of a translocation between chromosomes 4 and 5 since the offspring of a testcross to *cd; pe* consist of the following: mottled males and females, 1564; cardinal-peach males and females, 1341; and peach males, 1. In order to determine whether the break in chromosome 5 is distal or proximal to the *pe* locus, mottled males were crossed to wild-type females, and the resulting F_1 males were mated individually to peach females. The following types were found among the 2030 progeny produced from 14 F_1 males which carried the translocated chromosome inducing variegation: 52.6 percent wild type, 22.5 percent mottled, and 24.8 percent peach. The peach progeny can come either from nondisjunctional gametes formed in the $5/5^4/4^5/4$ F_1 males or from disjunctional gametes produced by F_1 males having the constitution $5/5/5^4/4$. In either case, the peach phenotype of such individuals indicates that the break is distal to the *pe* locus. Evidence corroborating this finding can be seen in the testcrosses to *cd; pe* mentioned above. If the break were proximal to the locus,

peach and cardinal-peach-mottled flies should have been observed in appreciable frequencies, but such was not the case. The fact that, in both these rearrangements it has been shown that the break is distal to the gene yet no abnormalities in the salivary chromosomes are observed, indicates that the distal limit of the region in which this locus is contained is the first of the four dark bands of basal heterochromatin. If the break were distal to this point, it could be detected easily.

The proximal limit of the region containing the *pe* locus can be deduced from crosses involving pe^{m4}. In crosses of this mottle to peach, a few abnormal flies having peach eyes are always produced. Cytological examinations of the ganglion and salivary chromosomes from progeny of these abnormal flies show that the tip of chromosome 3 is present in triplicate, i.e., the constitution of the abnormal flies is $5^3/3/3$. The fact that these flies, which arise from 3-1 segregation in the translocation heterozygote, are invariably peach in phenotype indicates that the break is proximal to the *pe* locus. Cytologically, the break is between the last band of heterochromatin and the centromere, thus marking the proximal limit of the region within which *pe* is contained.

Relationship between variegation and heterochromatin

Since it has been established that the *pe* locus is in heterochromatin, it is instructive to determine the euchromatic-heterochromatic relationships which can evoke variegation. In the case of rearrangements pe^{m1} and pe^{m3}, it is obvious that foreign heterochromatin from either the Y chromosome or the base of chromosome 4 will cause the mottling when placed distal to the locus. It is also evident from pe^{m15} and pe^{m34} that foreign heterochromatin from the Y chromosome or the 4H3 region, when located proximal to the last four bands of heterochromatin of 5 and thus proximal to the locus, will induce variegation (see plate I B). Euchromatin placed proximal to the locus will also induce variegation as made evident by peach-mottled numbers *4* (see plate I C), *11, 12, 16, 18, 19, 22, 24, 25, 31, 32, 33, 35, 36, 37, 38, 39, 41, 42* (see plate I D), *45, 46, 50, 51* and *53*. The pe^{m2} rearrangement is the only case so far detected in which the presence of foreign euchromatin distal to the locus is responsible for the variegation (see plate I E). It is thus apparent that a disruption of the normal chromosome continuity on either side of the *pe* locus by substitution of either foreign heterochromatin or euchromatin is capable of evoking variegation.

Distribution of breaks associated with variegation

It is clear from an examination of the rearrangements associated with variegation that all involve at least a single break on one side or the other of the 5H2 region. It is also evident that most of the rearrangements involve the relocation of euchromatin to the proximal side of the *pe* locus. In this regard it is interesting to see if the euchromatin responsible for the mottling is randomly distributed among the chromosomes. Figure 2 is a diagrammatic map showing the relative length of the salivary chromosomes and the points of

breakage of the rearrangements. If both the breakage points brought into juxtaposition to the 5H2 region and the other associated breaks in the rearrangements are considered, it can be shown that there is no evidence of nonrandom distribution throughout chromosomes 1, 2, 4 and 5. However, if only the breaks brought into association with the *pe* locus are considered, there is definite evidence of nonrandom distribution in chromosomes 2 and 3. For example, in the latter chromosome, three of the six breaks found are within seven bands of each other. This raises the possibility that either these regions are particularly susceptible to breakage or that they are unique regions which, when placed next to *pe,* affect the expression of this locus. It should also be noted that relatively few breaks associated with variegation are observed close to the

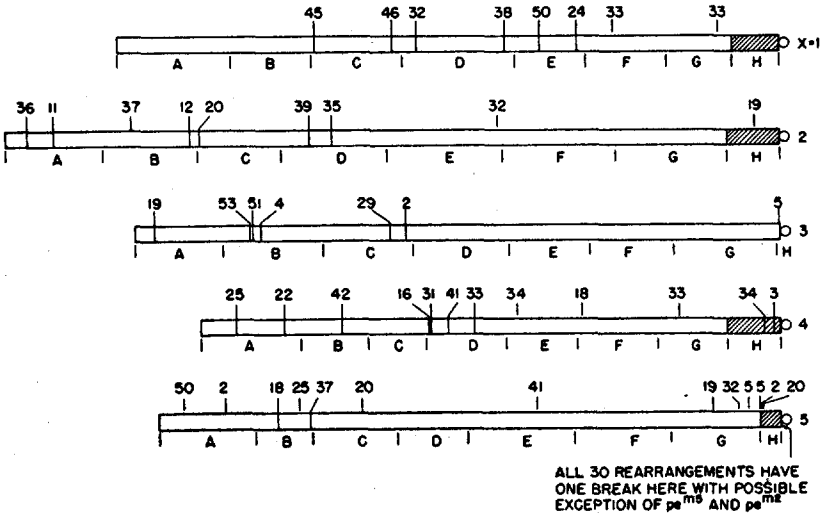


FIGURE 2.—Breakage distribution in salivary gland chromosomes of pe^{m} rearrangements. The shaded areas indicate the basal heterochromatic regions. For a given rearrangement, the line passing through the chromosome indicates the breakage point brought close to *pe,* the other lines indicate accompanying breaks.

proximal ends of the chromosomes. A similar nonrandom distribution of breaks associated with position effects at the *ci* locus in *D. melanogaster* was shown by KHVOSTOVA (1939) to be caused by the fact that other centromeric regions when placed close to *ci* did not influence the action of this gene.

It is evident that the great majority of the rearrangements involve a break between the last salivary band (5H2d) and the centromere. This fact took on added significance when it was discovered that rearrangements causing peach variegation were produced with an extremely high frequency by X-radiation. Pasadena males were given 5000 r and mated to *pe* females, and the F_1 progeny were examined for variegated eyes. Of the 969 F_1 males, 3 peach and 10 mottled males were observed, and of the 869 F_1 females, 22 had variegated eyes. It was possible to establish stocks of 8 of the mottles derived from the F_1 males and 14 of the mottles from the F_1 females. (It should be noted that,

in every case in which the eye pigmentation is variegated, a rearrangement has been detected either cytologically or genetically.) The original mottled flies were crossed to peach flies of the opposite sex, and a count was made of the offspring. It was found, by lumping the data from all rearrangements, that only 36 percent of the F_2 males carrying the translocation (phenotypically wild type or mottled) were actually mottled. Similarly, in the F_2 females 52 percent of the flies which carried the rearranged chromosomes actually showed a detectable variegation in the eye. Therefore, if an estimate of the frequency with which viable rearrangements capable of producing variegation is desired, the penetrance must be taken into consideration. From these figures, it is estimated that about 28 F_1 males and 62 F_1 females carried a rearrangement capable of producing variegation. To these 90 flies should be added the three peach mutants, since the two of these which were fertile showed a deficiency of the 5H2 region. Thus 93 : 1838 or 5 percent carried rearrangements capable of causing variegation, and in the great majority of these the break close to the 5H2 region is between the last band of heterochromatin and the centromere.

This high break frequency within a small region of the salivary chromosome makes it appear that this region is particularly susceptible to breakage by X-rays. However, a more typical picture of the cytological length of this heterochromatic region can be seen in the ganglion chromosomes. Plate 1 F is a photograph of a peach deficiency ($pe^{Df52f23}$) as seen in the salivary gland chromosomes. The deletion is less than one-thirteenth the length of the salivary chromosome. Plate I G shows a prometaphase configuration in the ganglion cells of the same individual. In these chromosomes the deficiency has reduced the chromosome length to about three-fifths its normal size. Therefore, it is apparent that the heterochromatin at the base of chromosome 5, as seen in the salivary glands, comprises from about a third to half the chromosome length in ganglion cells.

DISCUSSION

There seems little doubt that in many cases the variegated type of position effect is in some manner related to simultaneous changes in euchromatic and heterochromatic chromosomal regions. On the basis of work on variegation in *Drosophila melanogaster,* SCHULTZ (1941, MORGAN and SCHULTZ 1942) has set forth a series of general " rules," which appear to hold true for the majority of the then-studied cases of this type of position effect. The rules which are pertinent to this discussion are as follows:

1. Genes normally in euchromatic regions must be placed near heterochromatin in order for variegation to be expressed. The closer the gene is to heterochromatin, the more extensive the variegation.
2. Heterochromatic genes must be placed near euchromatin for the evocation of variegation.
3. In the case of euchromatic genes the addition of extra heterochromatin (either Y chromosomes or heterochromatic regions of the X chromosome or of chromosomes 2 and 4, as well as certain " puff " regions) to

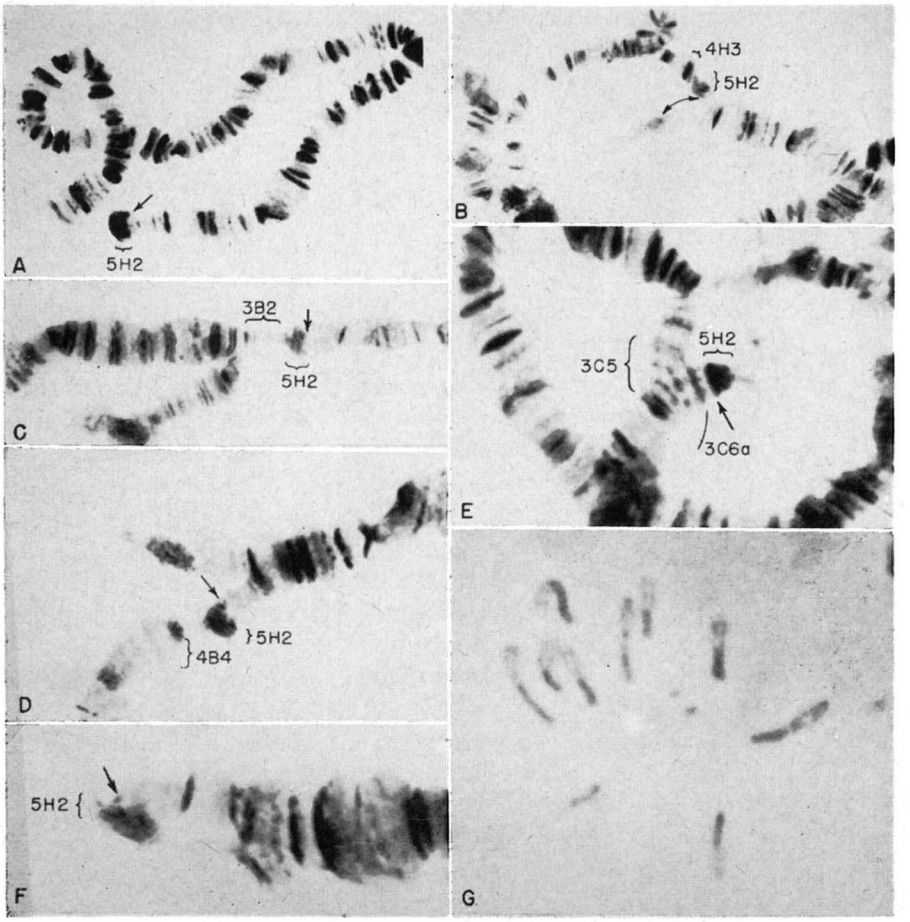


PLATE I

Chromosome rearrangements associated with variegation. In all the photographs the arrow points to the beginning of the 5H2 region within which the *pe* locus is contained.

A.—A normal chromosome 5.

B.—*T(4;5)pe*^{m34}. A portion of the basal heterochromatin of chromosome 4 is placed proximal to the *pe* locus.

C.—*T(3;5)pe*^{m4}. Euchromatin from 3 is located proximal to the *pe* locus.

D.—*T(4;5)pe*^{m42}. Euchromatin from 4 is placed proximal to the *pe* locus.

E.—*T(3;5)pe*^{m2}. Euchromatin from 3 is attached at a point distal to the *pe* locus.

F.—Deficiency *pe*^{Df52f23} as seen in salivary gland chromosomes.

G.—Ganglion chromosomes from the same larva as in F. The deficient chromosome is at eight o'clock.

the chromosome complement suppresses the variegation, whereas subtraction of heterochromatin enhances the mottling (see LEWIS 1950 for references).

4. With heterochromatic genes the addition of extra heterochromatin enhances the variegation (or further inactivates the gene), and its subtraction arrests the variegation.
5. When placed near heterochromatin, the euchromatic locus appears to be partially inactivated.
6. The homozygotes of rearrangements, which cause V-type position effects of euchromatic genes, have more extreme variegation than the heterozygotes.

It should be realized that, as far as the heterochromatic loci are concerned, the above rules are based predominantly on studies of a single locus, *lt* (light), located in the centromeric region of chromosome 2L. It is apparent that in *D. virilis* the apposition of a heterochromatic locus (*pe*) to euchromatin is a sufficient but not a necessary condition for the expression of variegation, since foreign heterochromatin will produce mottling equally well. If this observation proves to be generally applicable to heterochromatic loci, then it is evident that variegation is not only dependent upon partial inactivation of a gene brought about by bringing foreign chromatin close to its locus, but it also depends on disrupting the integrity of the heterochromatin. Viewed in this light, there may be no fundamental difference between the factors which evoke variegation in euchromatic and heterochromatic genes. A disruption of the continuity of the heterochromatic regions may be sufficient to produce the mutant phenotype of genes normally located in these regions or in those loci brought close to heterochromatin by rearrangements.

This interpretation of the results may seem at variance with the findings of KHVOSTOVA (1939) at the *ci* locus. She found that a change in action of *ci* is associated with its removal from heterochromatin immediately adjacent to spindle fiber attachment regions. Apparently other basal heterochromatin not immediately adjacent to the centromere, distal regions of the Y chromosome, as well as euchromatic regions are effective in modifying the expression of *ci*. These observations, in conjunction with the finding that other centromeric regions when placed close to *ci* did not produce a position effect, would seem to indicate a difference in the response of heterochromatic and euchromatic loci to a change in position. However, as pointed out by KHVOSTOVA (*loc. cit.*), the regions very close to the centromere may be homologous in all chromosomes. Therefore, rearrangements involving two such regions may not really disturb the continuity of the heterochromatin. It may be that an altered expression of genes located close to the point of breakage is observed only when different heterochromatin or when euchromatin is inserted into the basal heterochromatin.

It will be noted that there are three exceptions among the 32 rearrangements to the general observation that the variegation was accompanied by a break in the heterochromatin between the *pe* locus and the centromere. Al-

though in two cases (pe^{m1} and pe^{m3}) the break in chromosome 5 was distal to *pe*, the break in the other chromosome was in basal heterochromatin. In the third exception, pe^{m2}, one break in chromosome 5 was just distal to the locus while the other breaks were in euchromatin far removed from centromeric regions. The fact that in all the rearrangements showing *ci* position effects studied by KHVOSTOVA the break in chromosome 4 was invariably between *ci* and the centromere, raises the possibility that unobservable inversions in the heterochromatin of chromosome 5 might have accompanied these rearrangements and have been responsible for the appearance of the mutant phenotype in somatic tissue.

Theoretically, the mutant phenotype could be brought about either by a suppression of the gene products of the wild-type allele or by actual mutation or loss of the locus *per se*. Although cases of variegation have been interpreted by some workers to be caused by genetic instability in somatic cells, the stronger evidence points to a somatic disturbance of gene action (BELGOVSKY 1946; GERSH 1952; discussion by LEWIS 1950).

BELGOVSKY (1946) presents the hypothesis that variegation is caused by the interaction of substances produced by affected loci with those produced in heterochromatic regions. It is difficult to see how an interaction of products could explain the results, although a model based on interaction between substrates necessary for normal functioning of heterochromatin and genes is not unreasonable. For example, it might be assumed that the heterochromatin, at least within one region, acts as a unit in the formation of certain cellular constituents, P_H. Certain of the intermediates, I_H, in the formation of products from heterochromatin, require a substrate, X, for completion of a step in the reactions. If it is assumed that X can react with another substrate, Y, which is necessary in the reactions proceeding from the gene to end-product formation, e.g., precursors of the eye pigments, then any increase in the amount of X that can react with Y causes a decrease in the end product of the gene. Formally, this scheme may be diagrammed as follows:

$$\begin{array}{lllll} \text{Heterochromatin} \longrightarrow & I_H + X & \longrightarrow & P_H \\ & + & & \\ \text{Gene} \longrightarrow & I_G + Y & \longrightarrow & P_G \\ & \downarrow & & \\ & P_{XY} & & \end{array}$$

Thus, any disturbance in the integrity of the heterochromatin would cause less I_H to be formed, allowing X to react with any Y in its immediate vicinity. If the amount of available Y is a limiting factor in the amount of P_G produced, then this amount may approach the threshold value which corresponds to inactivation of the gene concerned.

The author is aware that such a general model is only one of many possible schemes on which the variegation data can be interpreted. Therefore its validity

is unknown and perhaps even doubtful. However, it serves to focus attention on the important problem of interactions between nuclear materials. For example, this scheme predicts that, if extra heterochromatin could be brought into a chromosome complement, then more I_H, and thus more P_G, might be produced which could result in a suppression of variegation. This prediction is apparently fulfilled except for the generalization by SCHULTZ that addition of heterochromatin enhances the variegation involving heterochromatic loci. So far as the author is aware, this generalization is based solely on the effect of different numbers of Y chromosomes on the expression of variegation at the light locus (MORGAN *et al.* 1934; MORGAN and SCHULTZ 1942) and possibly at cubitus interruptus (KHVOSTOVA 1939). MORGAN and SCHULTZ (1942) found that the addition or subtraction of a Y chromosome was without effect in the R(+) rearrangements involving rolled, a locus in the heterochromatin of 2R. The addition of an extra Y to the chromosome complement of the pe^{m1} rearrangement (a Y;5 translocation and thus not strictly comparable to the rearrangements at light) has no striking effect on the extent of mottling, although there may be a slight suppression. It should be noted that, according to the model, the addition of extra heterochromatin would be effective in suppressing variegation only if the additional I_H produced could increase the supply of Y by depleting the amount of X present in the *immediate vicinity* of the gene. In certain cases the presence of an extra Y apparently does not affect the variegation of euchromatic genes, e.g., yellow-mottled in *D. virilis* (GIRVIN 1949). SCHULTZ's observation that R(+)/R(+) genotypes show more extensive variegation than the rearrangement heterozygous with the mutant is difficult to explain on this scheme, unless it is assumed that heterochromatin produces some product which is essential in the reactions leading from gene to the production of its end product. None of the mottles which have been obtained in the homozygous condition (*3, 4, 31* and *39*) consistently show more extreme variegation in homozygotes than in heterozygotes.

There is one other line of evidence which lends some credence to the assumption that destruction of the continuity of the heterochromatin is just as necessary a factor in producing variegation as the juxtaposition of a gene to foreign chromatin. It was noted in the preceding section of this paper that most of the induced breaks associated with peach variegation occurred between the last band of salivary heterochromatin and the centromere of chromosome 5. It was also shown that the breakage frequency in this region is very high, owing presumably to the fact that this salivary region is greatly expanded in normal mitotic chromosomes. Unless the unlikely assumption is made that all these breaks are occurring at a localized spot close to the peach gene, it means that breaks anywhere within this relatively long region (which cannot be discerned in the salivary chromosome 5) are effective in evoking variegation. Studies are being made of the crossover frequency between *pe* and the point of breakage in different mottles as well as the distribution of heterochromatin in various rearrangements as seen in the prometaphase stage of ganglion cells. This work should provide direct evidence on the point under discussion.

If it is true that heterochromatic loci may be affected by breakage events taking place at some distance, then the situation is analogous to the spreading effect frequently observed with euchromatic genes. In terms of the gene-action interpretation of variegation, it means that there may be an interaction lengthwise along the chromosome between substrates utilized by the gene and those utilized by heterochromatin. However, this interaction is not effective between homologues because, as DUBININ and SIDOROV (1935) discovered, using the hairy (*h*) locus, R(+)/*h* flies showed partial expression of the mutant condition, but R(*h*)/+ (derived by crossing over from the former) flies were wild type. From crossovers derived from the latter genotype, the wild-type allele can be restored to the rearranged chromosome, and the mutant expression again becomes evident. This orientation along the length of the chromosome limits the interaction to factors localized on the same homologue. The orientation is thus similar to that proposed by LEWIS (1951) for explaining the stable-type position effect, thus suggesting at least one similarity between the two types of position effect.

SUMMARY

Thirty-two R(+) rearrangements induced by X-rays in *Drosophila virilis* are described which produce variegated pigment in the eyes when heterozygous with the recessive allele of the *pe* locus. This gene is the closest known gene to the centromere of chromosome 5, and evidence is presented that its locus resides within the last four bands of basal heterochromatin as seen in the salivary gland chromosome.

Among these rearrangements, examples are found which indicate that any one of the following types of association between euchromatin and heterochromatin is sufficient to induce variegation: (1) placing foreign heterochromatin distal to the *pe* locus, or (2) proximal to the locus; (3) placing euchromatin either distal or (4) proximal to the locus.

The great majority of the rearrangements involve a break in chromosome 5 between the last salivary band of heterochromatin and the centromere. The high break frequency observed in this region is probably due to the demonstrated fact that the small salivary heterochromatic region comprises at least two-fifths of the length of the normal mitotic chromosome.

It is held that these findings provide evidence that there may be no great difference in the reactions of heterochromatic and euchromatic loci to V-type position effects. Thus, for variegation to be evoked, it is necessary not only that the locus be brought near heterochromatin but also that the continuity of the heterochromatin concerned be interrupted. An interpretation of variegation is given in terms of interaction between necessary substrates of gene and heterochromatin.

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