DIRECT PROOF OF A VARIEGATED-TYPE POSITION EFFECT AT THE WHITE LOCUS IN DROSOPHILA MELANOGASTER[1]

BURKE H. JUDD[2]

Genetics Foundation, Department of Zoology, University of Texas, Austin, Texas

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VARIEGATED-type position effect in *Drosophila melanogaster* has received a considerable amount of attention from many investigators (See review by LEWIS 1950). Direct proof of this phenomenon has been obtained for only two cases however. DUBININ and SIDOROV (1935) showed that the action of the gene for hairy (*h*) was affected when this locus was placed near heterochromatin of the fourth chromosome by a translocation between the third and fourth chromosomes. PANSHIN (1935) studying another 3-4 translocation showed that the curled gene (*cu*) also became unstable when placed near heterochromatin. By taking advantage of a small amount of crossing over between the variegating gene and the break point of the translocation, these workers were able to show in both cases that the gene in question behaved as the standard + allele when placed in a chromosome of normal structure. Proof of the position effect came from a comparison of translocation heterozygotes that differed in the position of the + and mutant genes. Variegation occurred in heterozygotes having the + allele in the rearranged chromosome and the mutant allele in the normal homolog, while heterozygotes in which the position of these two alleles had been exchanged gave a wild type phenotype. It was shown that the variegation is not the result of structural heterozygosis but depends only on the position of the + allele.

The present investigation provides direct proof of variegated-type position effect for the white locus. White-mottled types have been widely studied but only indirect evidence for position effect has been provided (SCHULTZ 1936; SACHAROV 1936; DEMEREC 1941; and others). The white gene provides more satisfactory material for working out this direct proof than does either curled or hairy. DUBININ and SIDOROV and particularly PANSHIN were dependent on the phenotype of a population of flies in order to determine the presence or absence of variegation. In the case of white, variegation, or the absence of it, can be detected easily in a single individual since there are some 800 facets in each eye and a change of color in only a few facets can easily be seen.

EXPERIMENTAL

The X-4 translocation $w^{258\text{-}21}$ (BRIDGES and BREHME 1944) was used for this study. The region to the left of 3E5 in the X chromosome has been moved to the heterochromatic region near the centromere of chromosome 4. The X chromosome genes

[1] This work is part of the thesis submitted to the faculty of the California Institute of Technology in partial fulfillment for the degree of Doctor of Philosophy.

[2] Presently, Fellow in Cancer Research of the American Cancer Society, Genetics Foundation, Department of Zoology, University of Texas, Austin, Texas.

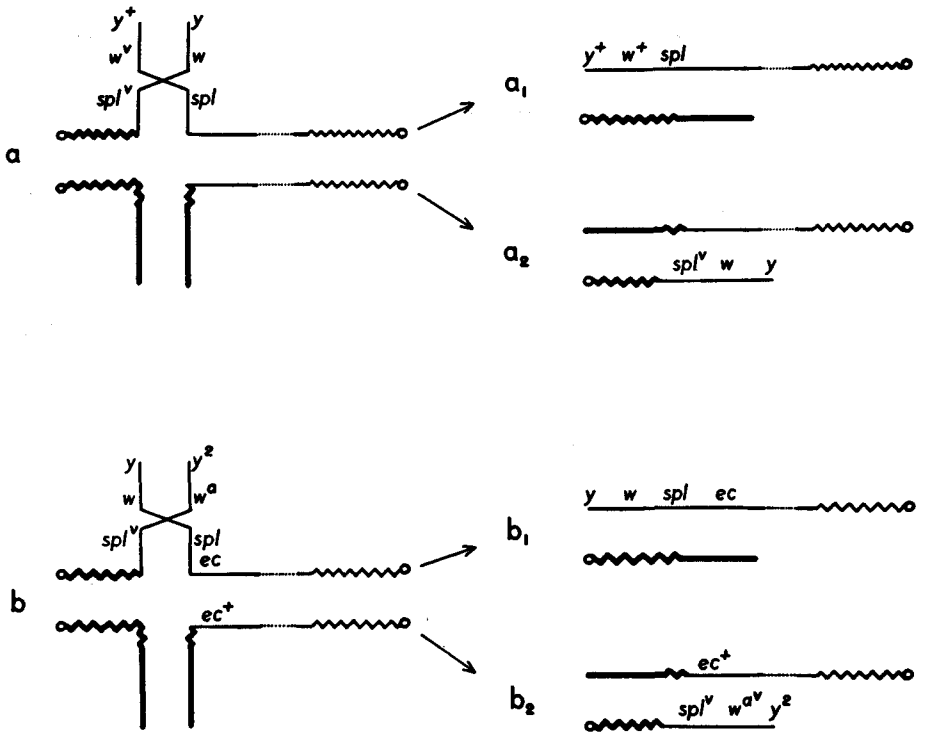

FIGURE 1.—Diagrammatic representation of $w^{258\text{-}21}$ heterozygotes, showing pairing relationships. The positions of the genes yellow (*y*), white (*w*), split (*spl*), and echinus (*ec*) are shown; variegation of a gene is indicated by the superscript *v*. The X chromosome is represented by the lighter lines while the heavier lines represent chromosome 4. Euchromatin is indicated by the straight lines and heterochromatin is shown by the zigzag lines. The centromeres are shown as open circles. A crossover between the white locus and the translocation break point is indicated and the resulting products are shown.

diminutive (*dm*), facet (*fa*), split (*spl*), and white (*w*) show variegation in flies heterozygous for the translocation and the mutant gene; echinus (*ec*) shows no variegation. This translocation also shows a variable Notch (*N*) phenotype and is lethal in the hemizygote.

The proof of position effect is based on the recovery and analysis of chromosomes in which a crossover has occurred between the white locus and the break point of the translocation. The basic experimental set-up is shown diagrammatically in figure 1. Females were obtained heterozygous for the translocation and a structurally normal X chromosome carrying the mutant genes yellow (*y*), white (*w*), split (*spl*), and singed-3 (sn^3) (fig. 1a). The second and third chromosomes were made heterozygous for chromosomal rearrangements since such an autosomal condition is known to be effective in increasing crossing over in the X chromosome (STEINBERG 1936). Two Curly (*Cy*) inversions (See BRIDGES and BREHME 1944) were used for the second chromosome. The left arm is marked by the dominant Curly (*Cy*), while the right arm carries speck-2 (sp^2). For the third chromosome the complex rearrangement Ubx^{130} (*Ubx* = Ultrabithorax, a dominant bithorax-like

change, LEWIS 1952) was used. These females were mated to *y w spl sn*^{3} males and the offspring were examined for crossing over between *w* and *spl*. Two females of the phenotype *y w spl*$^+$ were found in approximately 22,000 female offspring examined. One of these females proved fertile, and gave *y w* and *y w spl* daughters and *y w spl* sons when mated to her *y w spl* sibs. A stock of the translocation now containing *y* and *w* (represented as product a_2 in fig. 1) was obtained by balancing with *In(1)sc*^{8}, *dl-49*, *y*^{31d} *w*^{a} *lz*^{s} (lozenge-spectacled) *B* (Bar). Females from the stock were crossed to *y* males and slides were made of the salivary glands from the *y* larvae. Cytological examination of the salivary gland chromosomes showed an X-4 translocation to be present with the breaks at the same points as described for the original *w*$^{258\text{-}21}$.

The complementary crossover class, diagrammed as product a_1 in figure 1, was also recovered. Two males of this class were found; both had normal wild type body and eye color (*y*$^+$ *w*$^+$). One carried *spl* while the other was *spl*$^+$; both were *sn*^{3}.

Since R(*w*)/*w*$^+$ flies prove to have a wild type eye color, as discussed below, it was felt that a more sensitive test for detecting the possibility of variegation in the heterozygote containing R(*w*) would be to employ a standard chromosome carrying an intermediate "allele" of white instead of *w*$^+$. The pseudoallele, (LEWIS 1952) apricot (*w*^{a} or *apr*), was chosen for this purpose. The genotypes to be compared for a precise position effect test were therefore R(*w*)/*w*^{a} and R(*w*^{a})/*w*. For this reason the construction of R(*w*^{a}) was undertaken.

Part b of figure 1 shows the X chromosome constitution of the females used for this experiment. Females were obtained heterozygous for the translocation which carried *y w* and a normal chromosome having the mutant genes *y*^{2} *w*^{a} *spl ec*; they were mated to males carrying *y w ec f* (forked). In the first of these matings the same autosomal rearrangements described above were used. No crossover products of the desired type were found in about 250 cultures examined and so other autosomal rearrangements were introduced. In one of the second chromosomes the rearrangement *SM1*, *al* (aristaless) *Cy sp*^{2} was used and the homolog carried *In(2LR)102*, *ds*^{w} (dachsous-Wide) *sp*^{2} (according to LEWIS and MISLOVE, personal communication, *SM1*, or "Second Multiple 1," refers to a complex of inversions consisting of a pericentric inversion with breakage points in 22A and 60B, superimposed on a chromosome carrying both left and right arm Curly inversions, while *In(2LR)102* has the following sequence: 21A to 26A/51D to 41/57A to 51D/26A to centromere of 2 to 41/57A to 60F). One of the third chromosomes contained *Ubx*^{130} and the other contained *In(3LP, 3RC) Sb e*^{s} (ebony-sooty). From this experiment two females with the phenotype yellow-2, dilute-apricot (*w*^{a}/*w*) were found among approximately 3,200 females examined. Both of these were the result of crossing over between *w* and *spl* and are represented in figure 1 as product b_2. Cytological examination of the salivary gland chromosomes confirmed the presence of the translocation.

The complementary crossover class was also recovered during these experiments. A total of five such crossovers were found, however, the total number of individuals examined was not recorded. Four of the crossovers occurred between *w* and *spl*; one took place between *spl* and the translocation break point.

A crossover between *ec* and the translocation break point would give the same phenotype (yellow-2, dilute-apricot) as the sought-after class; however, only three bands on the salivary gland chromosome map (BRIDGES 1938) separates these two points. Three females in which this type of crossover had occurred were picked up during these experiments. Males of this type would not be noticed since they would be y^2 w^a *spl* ec^+, thus indistinguishable for the most part from their y^2 w^a *spl ec* brothers.

To test the position effect in $w^{258\text{-}21}$, experiments were set up to compare the original translocation, R(w^+), with the newly substituted R(w). The variegation is known to be modified by the presence of an extra Y chromosome and by the temperature at which the culture is grown (GOWEN and GAY 1933, 1934). Therefore, single females from the R(w^+) and R(w) stocks were outcrossed to *In (1) dl-49*, *y Hw* (Hairy wing), m^2 (miniature-2), g^4 (garnet-4) males. Secondary non-disjunction in any of the females could be detected by this cross and in this way any XXY females could be eliminated. The R(w^+)/*In (1) dl-49*, *y Hw* m^2 g^4 offspring were mated to *y w ec f* or *y w spl* sn^3 males. The R(w)/*In (1) dl-49*, *y Hw* m^2 g^4 females were crossed with w^+ *spl* sn^3 or w^+ spl^+ sn^3 males, both types of which were described earlier as being derived by crossing over from the original translocation. One third of each of the two types of cultures were placed at 25°C, one third at 19°C, and the remaining one third were grown at 14°C. Each culture bottle was given a code number so that the genotype was not known until all the results had been tabulated. It was found that R(w^+)/w showed variegation for white; this was very marked in those cultures grown at 19°C and 14°C. The cultures of type R(w)/w^+ did not show mottling for white even when grown at these lower temperatures; although, as expected, both types of cultures showed variegation for split when the mutant *spl* was present in the chromosome of normal structure. All flies heterozygous for the translocation showed variegation for the dominant Notch; again this was more extreme in cultures grown at the two lower temperatures. To test the R(w) rearrangement further for the occurrence of variegation, females heterozygous for R(w) and each of the white alleles apricot (w^a), buff (w^{bf}), cherry (w^{ch}), coral (w^{co}), eosin (w^e), honey (w^h), ivory (w^i), satsuma (w^{sat}), and tinged (w^t), were cultured at 19°C. No mottling was observed in the eyes of any of these females, while females heterozygous for these alleles and R(w^+) showed mottling in every case.

When the R(w^a) type was obtained, somewhat similar comparisons were made. R(w^a)/w gives a mottled pattern consisting of white or near-white spots on a dilute-apricot background, while R(w)/w^a gives a non-variegated dilute-apricot phenotype. R(w^a)/w^+ is wild type in phenotype with no indication of mottling. Variegation for split occurs in every case when *spl* is in the normal homolog.

DISCUSSION

The results from these experiments show conclusively that the somatic variegation in the expression of the white gene in the translocation $w^{258\text{-}21}$ is due to a position effect. The w^+ gene of the original translocation shows a variegated phenotype in the eyes of females heterozygous for the rearrangement and w. When this w^+

allele is transferred to an X chromosome of normal structure this variegation is lost and the gene functions as the dominant wild type allele. When the mutant gene *w* is placed in the translocation, no variegation is detected in flies of the type R(*w*)/*w*$^+$, even under low temperature culture conditions. When the intermediate pseudoallele of white, *w*^{a}, is placed in the translocation its action is affected such that R(*w*^{a})/*w* flies give a mottled phenotype while R(*w*)/*w*^{a} flies show no trace of variegation.

The conclusion indicated by these facts is that a variability of action is conferred on the gene in the structurally abnormal chromosome while the allele in the structurally normal homolog retains its usual activity. The mutant allele *w* would not be expected to show any variability since the mutant gene itself is characterized by its loss of activity, that is, the same phenotype is exhibited by a deficiency for the white locus. The variegation in these cases must, therefore, be the result of the inactivation of the gene or its product when the gene in question is located in the rearranged chromosome.

This conclusion is not in agreement with the interpretation of the position effect phenomenon put forth by EPHRUSSI and SUTTON (1944) and supported by MULLER (1941, 1947). This is a structural hypothesis based on the assumption that the shape of the gene rather than its chemical composition may be altered in a way which is readily reversible. Variegation is interpreted as being the result of the upset of pairing relationships of the regions adjacent to the breaks of a chromosomal aberration in a heterozygous individual. A change in stress upon the gene might be expected to alter its activity. By this hypothesis, variegation should occur in R(*w*)/*w*$^+$ flies as well as the R(*w*$^+$)/*w* and R(*w*^{a})/*w* types. The pairing relationships are expected to be precisely the same in these three genotypes, therefore, the variegation exhibited only by the two latter types cannot be due to structural heterozygosity alone. LEWIS (1950) has pointed out that the type of evidence presented here serves to dispose of the interhomolog influence in this type of position effect, which points up even more clearly the intrachromosomal position effect, in particular the euchromatin-heterochromatin association, as the fundamental problem to be considered.

SUMMARY

The variegation for white exhibited by the translocation *w*$^{258\text{-}21}$ is shown to be due to variegated-type position effect. The variegation is dependent on the variable effect of the allele placed in the proximity of heterochromatin in the structurally rearranged chromosome and is independent of the allele in the structurally normal homolog.

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