CYTOGENETIC STUDIES ON THE WHITE LOCUS IN *DROSOPHILA MELANOGASTER*¹

G. LEFEVRE, JR. AND M. D. WILKINS

*The Biological Laboratories, Harvard University, Cambridge, Massachusetts*²

SOME years ago, a favorite preoccupation of *Drosophila* cytogeneticists was locating specific genes on the salivary chromosome map. The results of their labors, which in large part have been recorded by BRIDGES and BREHME (1944), led to the conclusion that, in general, individual salivary chromosome bands contain individual gene loci. Only in the case of certain thick bands have two or more nonallelic genes been found to occupy one and the same salivary band (MULLER and PROKOFYEVA 1935). On the other hand, in some instances, genes appeared to extend over more than one band. From these considerations, among others, the total number of independent gene loci in *Drosophila* was estimated to be of the same order of magnitude as the total number of salivary chromosome bands, or about 5,000.

Today, as a result of biochemical investigations, it is not unreasonable to believe that the total number of different genes in *Drosophila* may greatly exceed 5,000. Although some gene-containing bands may be too delicate to be resolved with the light microscope, it should be profitable to reinvestigate the localization on the salivary chromosome of genes that appear to extend over more than one band. Of such genes, perhaps none is more appropriate for study than is the white locus.

At the white locus, numerous phenotypically distinguishable alleles (BRIDGES and BREHME 1944) and at least five recombinational sites (JUDD 1964) have been identified. If, as LEWIS (1952) has suggested, the subunits of the white locus originated by tandem duplication of an ancestral locus, then it would indeed seem plausible to suppose that the white locus extends for some distance along the physical chromosome, perhaps spanning more than one salivary band.

In point of fact, the white locus has been associated by one investigator or another with salivary chromosome bands 3C1, 3C2, and 3C3 of BRIDGES' (1938) map. The case for its association with band 3C1 seems especially strong. Several deficiencies of the white locus have been reported to lack band 3C1, but not bands 3C2 and 3C3. Most pertinent is the male-lethal white mutant w^{258-45} , analyzed by SUTTON and described in BRIDGES and BREHME (1944), which was said to be deficient only for band 3C1. In the absence of other evidence, the existence of w^{258-45} would seem to restrict the white locus to band 3C1.

An equally strong case can be made, nonetheless, for the proposition that the white locus is contained within the double band 3C2-3, and does not extend into 3C1. The evidence for this derives from the result of crossing over between two

¹ This investigation was aided by research grants from the Public Health Service (GM-07667) and from the National Science Foundation (GB-2097).

² Current address: Department of Biology, San Fernando Valley State College, Northridge, California.

similar X-chromosome inversions, white-mottled-4 (w^{m4}) and roughest-3 (rst^s). A single crossover within the mutually inverted regions of w^{m4} and rst^s gives rise, in one direction, to a deficiency for the white locus (PANSIN 1941). From the known positions of the euchromatic breakpoints of the two inversions, we can infer that the crossover chromosome which bears the left end of w^{m4} and the right end of rst^s (and which lacks the white locus) is deficient for bands 3C2-3, but not for 3C1. Thus, the two lines of evidence bearing on the salivary chromosome position of the white locus seem incompatible. Nonetheless, females heterozygous for w^{258-45} (lacking 3C1) and $w^{m4L}-rst^{sR}$ (lacking band 3C2-3) are viable and white-eyed (GREEN 1959a).

The existence of viable, white-eyed females whose chromosome constitution is Df 3C1/Df 3C2-3 is paradoxical. Their survival implies that they are not homozygous deficient for essential genetic material; yet they are white eyed. The conclusion that such females must be homozygous deficient for the white locus seems inescapable, despite the cytological evidence to the contrary. GREEN (1959a, 1963) has concluded that the array of white pseudoalleles is confined to the double band, 3C2-3; more specifically, that white alleles which act as suppressors of the eye-color mutant *zeste* (*z*) are localized in 3C3 and that nonsuppressors of *z* are in 3C2.

Without additional pertinent mutants for study, analysis beyond this point is virtually impossible. Recently, however, several new mutants affecting the white locus have arisen during the course of irradiation experiments in our laboratory. Following a careful cytogenetic analysis of these new mutants, we are now able to identify more precisely the salivary chromosome position of the white locus and to resolve the ambiguities of the earlier evidence.

THE EXPERIMENTAL CHROMOSOMES

The salivary chromosome position of the white locus has been investigated through the use of several different aberrant X chromosomes containing breaks at or near the white locus. These abnormal chromosomes include inversions, deficiencies, and duplications affecting the white locus. Crosses between them, in conjunction with an extensive salivary chromosome analysis of each, have permitted decisive inferences to be drawn regarding the position of the white locus. In addition, the white allele, spotted-white, was employed to test for the presence or absence of the white locus in the experimental chromosomes. Some of these have long been available and will be described briefly; others are new and will be described in greater detail.

1. *spotted-white* (*sp-w*): an unusual white allele with a relatively light, nonuniform distribution of eye pigment. Unlike any other white allele, *sp-w* complements with *w* alleles of mutational origin, giving a dark (but not wild-type) eye color in *sp-w/w* females, but not with true deficiencies for the white locus (GREEN 1959b; JUDD 1961a).

2. *white-258-45* (w^{258-45}): an X-ray-induced male-lethal white mutant produced by DEMEREC which is deficient only for band 3C1, according to SUTTON (in BRIDGES and BREHME 1944). Our stock of this mutant, which was received from JUDD and which was used in the present studies, has acquired a second short deficiency just to the left of 3C1. In heterozygous combination with

deficiencies of 3C2-3, w^{258-45} gives rise to viable, white-eyed females; but it does not complement with $sp-w$ (GREEN 1959b).

3. *Deficiency of white through echinus* ($Df(w-ec)^{64d}$): a new X-ray-induced deficiency, extending from the white locus through the echinus locus; cytologically, from 3C2 through 3E7, inclusive. The deleted segment was simultaneously inserted in section 37D of Chromosome 2. Males containing both the deficiency and the insertion are viable, express both the white and echinus mutant characters, and have normal wings. Females heterozygous for $Df(w-ec)^{64d}/w-ec$ are white-eyed, echinus, and Notch. Neither the deficiency nor the insertion complements with $sp-w$.

4. *white-marbled Notch-63b* ($w^{ma} N^{63b}$): a new X-ray-induced Notch deficiency extending from 3C3 through 3E2, inclusive, which is accompanied by a simultaneously induced, nonuniformly pigmented white allele, which we have called "marbled." The eye color expressed by $w^{ma} N^{63b}/w$ is quite similar to that exhibited by $sp-w/w$ complementing heterozygotes. However, $w^{ma} N^{63b}/w^{258-45}$, which survives, does not have the noticeably lighter eye color characteristic of $sp-w/w^{258-45}$ females.

5. *white-Variegated cobbled* (w^{Vco}): an insertional translocation between the X chromosome and Chromosome 3, discovered by CLAUSEN. According to SCHULTZ (in BRIDGES and BREHME 1944), a piece of the X chromosome from 2C1 through 3C4 has been inserted in the heterochromatic region of Chromosome 3, which itself has a short inversion across the spindle attachment region. As a duplication, w^{Vco} exhibits strong variegation for both white and roughest, but covers deficiencies of 3C1 and 3C2-3; that is to say, w^- ; Dp w^{Vco} males survive with strongly variegated eyes.

6. *Duplication w^{+51b7}* (Dp(1;2R) w^{+51b7}): a short segment of the X chromosome, produced by the irradiation of w^{m4} , extending from 3D5-6 through 3C2, and doubtless including some adjacent proximal heterochromatin (but not 3C1), has been inserted in the euchromatic region of the right arm of Chromosome 2 at 54F (LEFEVRE 1952; RATTY 1954). No variegation is exhibited for white, roughest, or split, all of which are included in the duplication, despite the fact that the duplication was derived from w^{m4} . Dp w^{+51b7} covers all white alleles, in the sense that w^x ; Dp w^{+51b7} males are completely wild type in eye color, but it does not cover the lethality of w^{258-45} (GREEN 1959a).

7. *white-mottled-4* (w^{m4}): a long inversion of the X chromosome, discovered by MULLER. According to SUTTON (in BRIDGES and BREHME 1944), the left breakpoint is between salivary chromosome bands 3C1 and 3C2. The right breakpoint is in the proximal heterochromatin to the left of bobbed (LINDSLEY 1954). Genetic studies have shown that the white locus is included in the inversion, so that it is in association with the uninverted proximal heterochromatin. (See Figure 1.) Males and homozygous females are viable and fertile, and their eyes exhibit typical white-variegation, which is suppressed by the presence of an extra Y chromosome.

8. *roughest-3* (rst^3): an X-ray-induced long inversion of the X chromosome, discovered by GRÜNEBERG. According to EMMENS (1937), the left breakpoint follows 3C3, and the right breakpoint, as in w^{m4} , is in the proximal heterochromatin to the left of bobbed (LINDSLEY 1954). In rst^3 , the white locus is not inverted. Males and homozygous females are viable and fertile, and their eyes exhibit a rough texture, resulting from disarranged facet rows. There is no evident variegation for white in XY rst^3 males, despite the proximity of the white locus to the inverted segment of heterochromatin. (See Figure 1.)

9. *white deficiency derived from crossing over between w^{m4} and rst^3* ($w^{m4L-rst^3R}, w^-$): This deficiency includes only bands 3C2-3, and behaves as a male-lethal white mutant which fails to complement with $sp-w$ (GREEN 1959b). It is covered by Dp w^{+51b7} and by Dp w^{Vco} , as would be expected. Females heterozygous for $w^{m4L-rst^3R}, w^-/w^{258-45}$ are fertile, viable, and white-eyed, but produce no sons.

10. *white-mottled-J* (w^{mJ}): a new long inversion of the X chromosome, produced by irradiation, whose left breakpoint is just to the right of the white locus (*Drosophila Inform. Serv.* 37: 49). Thus, unlike the situation in w^{m4} , in w^{mJ} the white locus itself is not inverted. The right breakpoint is in the proximal heterochromatin well to the left of bobbed. Further, in w^{mJ} the inverted X chromosome is no longer borne on the X centromere, but has become translocated

onto Chromosome 4. The fourth-chromosome breakpoint, in the euchromatic region at 102C, separates the eyeless and cubitus-interruptus loci. (See Figure 1.) Despite the fact that the white locus is not inverted in w^{mJ} , the eyes exhibit marked variegation for white, but not for roughest. Because of the translocation, there is an extensive deficiency of proximal heterochromatin including the bobbed locus and the nucleolar organizer, which results in the death of XO w^{mJ} males. Homozygous w^{mJ} females survive with poor viability only with an extra Y chromosome. Heterozygous w^{mJ}/w^{m4} females exhibit to a slight degree white-mottled variegation.

RESULTS

1. *The evidence from w^{mJ}* : In general, a single crossover in the mutually inverted region of two similar, but not identical, inversions gives rise to a deficiency or a duplication of the genetic material between the corresponding breakpoints of the two inversions. Thus, as we have noted above, crossing over between w^{m4} and rst^3 results in a male-lethal white deficiency, which, from the known positions of their euchromatic breakpoints, must include only salivary band 3C2-3. (See Figure 2.) If this double band contains no genetic material other than the white locus, then the white locus must be described as vital to the organism and its homozygous loss incompatible with survival. Alternatively, band 3C2-3 may contain not only the white locus, but also one or more additional loci. Although the loss of the whole complex is lethal, the lethality would not necessarily imply

FIGURE 1.—Diagrammatic representation of wild-type, white-mottled-4 (w^{m4}), white-mottled-J (w^{mJ}), and roughest-3 (rst^3) mitotic X chromosomes, showing the relationships to heterochromatin of various gene loci: yellow (y , 0.0), white (w , 1.5), roughest (rst , 1.7), crossveinless (cv , 13.7), cut (ct , 20.0), vermilion (v , 33.0), forked (f , 56.7), carnation (car , 62.5), and bobbed (bb , 66.0). Note that the left breakpoint of w^{m4} is to the left of both the w^+ and rst^+ loci; whereas, in w^{mJ} and rst^3 , the left breakpoint is between the w^+ and rst^+ loci. The right breakpoints of all three inversions occur at approximately the same position in the heterochromatin.

FIGURE 2.—The results of reciprocal recombination between w^{m4} and w^{mJ} , between w^{mJ} and rst^3 , and between w^{m4} and rst^3 are illustrated. Note that a single crossover within the mutually inverted segments of the inversions gives rise, in one direction, to a duplication of the euchromatic material between the left breakpoints, and in the other direction to a deficiency of the same material. The salivary chromosome position of the euchromatic breakpoints is indicated.

that the white locus itself is required for survival. MULLER (1935), for example, has shown that homozygous deficiency of yellow and achaete is not lethal.

In the absence of additional inversions similar to w^{m4} and rst^3 , no greater resolution of this problem is possible. By chance, as a by-product of studies on the frequency of X-ray-induced mutations, just such a new inversion arose in our laboratory. This inversion, w^{mJ} , exhibits white variegation and generates duplication and deficiency crossover products with both w^{m4} and rst^3 . Of particular significance is the fact that females heterozygous for w^{m4}/w^{mJ} (which themselves have mottled eyes) readily produce by recombination w^{m4L}/w^{mJR} viable and fertile white-eyed sons. (See Figure 2.) When tested with $sp-w$, these white-eyed recombinants fail to complement, indicating the absence of the white locus. This result, by itself, demonstrates that the white locus is not required for survival, and further, it implies that part or all of the white locus is contained in the inter-

val between the euchromatic breakpoints of w^{m4} and w^{mj} . Females heterozygous for rst^3/w^{mj} , crossed with w males, produce no white-eyed progeny, but $w^{mjL}-rst^{3R}$ recombinants are male-lethal. (See Figure 2.) This lethality is covered by $Dp\ w^{+s1b7}$, which implies that in the interval between the left breakpoints of w^{mj} and rst^3 is a locus, independent of the white locus, that is required for survival.

The salivary chromosome constitution of w^{mj} has been extensively studied in males and in females heterozygous for wild-type, w^{m4} , and rst^3 . In addition, preparations of larval brain ganglion cells of $w^{mj}/+$ females have been studied. Photographs of representative cells are shown in Figure 3, in which the left breakpoint of w^{mj} does appear to be to the right of the left breakpoint of w^{m4} (Figure 3A). Genetically, the left breakpoint of w^{mj} must be to the right of the left breakpoint of w^{m4} , which itself is clearly between 3C1 and 3C2. Therefore, only the following possibilities exist for the position of the left breakpoint of w^{mj} : (1) between 3C1 and 3C2, but closer to 3C2 than is the left breakpoint of w^{m4} ; (2) through the substance of 3C2; (3) between 3C2 and 3C3; (4) through the substance of 3C3; and (5) between 3C3 and 3C4, but closer to 3C3 than is the left breakpoint of rst^3 . Of these possibilities, the first and fifth would require that genes such as white exist between microscopically visible salivary chromosome bands. The second and fourth possibilities require that single bands are subdivisible (that is to say, compound) and contain the loci of more than one gene. The third possibility, *i.e.*, that in w^{mj} the left breakpoint lies between bands 3C2 and 3C3, provides the most economical explanation for the results. Perhaps loci do reside in interband spaces; perhaps individual bands are subdivisible; but the evidence from the present study is insufficient to force such assumptions. As the simplest interpretation of the evidence from w^{mj} , then, the white locus is contained within band 3C2, which is not essential for survival, and an independent lethal locus lies in band 3C3.

2. *Supporting evidence from two other mutants:* Salivary chromosome cytology is an inexact science at best, and the precise identification of individual bands at chromosome breakpoints is always difficult. Moreover, the 3C region is notoriously refractory to detailed analysis. Thus, the assignment of the white locus to band 3C2 on the basis of the evidence from w^{mj} alone cannot be fully convincing. Fortunately, two additional mutants, both deficiencies which were also discovered in the course of mutation experiments, provide evidence to support the belief that the white locus is confined to 3C2.

The first of these mutants, $w^{ma} N^{63b}$, which is male-lethal and has been described above, is especially interesting. When first detected, it appeared to be a white-mottled Notch mutant of no unusual significance. Not until salivary chromosome preparations of this mutant were studied was its true character appreciated. Rather than being a translocation or an inversion, it proved to be a simple deficiency, as illustrated in Figures 4A and B. Closer examination of the eye-color phenotype of $w^{ma} N^{63b}/w$ females revealed that the pattern of pigment distribution was not typical of white-mottled mutants, but rather resembled to a remarkable degree the blotchy appearance of $sp-w/w$ heterozygotes. The salivary chromosome preparations, particularly in unsynapsed strands as illustrated in

FIGURE 3.—The cytology of *w^mJ*. A. Salivary chromosome of *w^mJ/w^m4* female. At the left breakpoint, note that band 3C1 remains uninverted in *w^m4*, but in *w^mJ* both 3C1 and 3C2 remain uninverted. B. *w^mJL*, unsynapsed and well-stretched, shows more clearly that 3C1 and 3C2 are not inverted. C. *w^mJ/+* female. Note the continuity between *w^mJR* and Chromosome 4. D. Late mitotic prophase in a *w^mJ/+* female larval brain preparation. Note the relatively small amount of inverted heterochromatin in *w^mJ*, and also the occurrence of an extra Y chromosome.

Figure 4A, indicate that bands 3C1 and 3C2 have not been deleted. The white locus obviously cannot have been deleted since the eyes are pigmented. The absence of band 3C3 can be inferred since females heterozygous for this mutant and $w^{mJL-rst^{SR}}$ (which we believe to be deficient only for 3C3) fail to survive.

We interpret this mutant as having resulted from a deletion of material immediately to the right of the white locus, material which includes the Notch locus. Whether the change at the white locus resulted from an induced mutation adjacent to the point of chromosome breakage or from a stable euchromatic position effect on the white locus cannot be decided. In any event, it is remarkable that the white-marbled allele, lying as it does just to the left of the Notch deficiency, exhibits a nonuniform distribution of eye pigment reminiscent of the phenotype of *sp-w*. Is it only coincidental that *sp-w* occupies the rightmost recombinational site of the white locus (Judd 1964), and thus is immediately adjacent to the point of breakage in $w^{ma} N^{esb}$?

The second mutant is even more unusual. Among the progeny of an irradiated wild-type male and a white-eyed female was a daughter that appeared to be simply a white-eyed mutant. On crossing with *w* males, this mutant produced sons whose eyes not only were white, but were also rough in texture. In addition, two classes of white-eyed daughters appeared: Notch and non-Notch. Salivary chromosome analysis revealed a short deficiency in the X chromosome (Figure 4C), accompanied by an insertional translocation of the deleted X-chromosome material into section 37D of Chromosome 2. The left X-chromosome break immediately follows 3C1 (as is the case in w^{m4}). The absence of the white locus from the deficiency is demonstrated by the fact that it fails to complement with *sp-w*. We should expect, then, a mutant white locus to be present in the translocated segment. This, however, seems not to be the case since $Df(w-ec)^{64d}$; $Dp(w-ec)^{64d}/sp-w$ females also fail to complement. Thus, the white locus (and presumably 3C2) appears to be missing from both the deficiency and the insertion, but this lack by itself is not sufficient to result in the death of duplication-deficiency males. The situation is precisely analogous to that of viable $w^{m4L}-w^{mJR}$, *w*⁻ males, deficient for 3C2. The rough-eyed character expressed by the white-eyed duplication-deficiency males is actually an expression of echinus, as was proved by crossing to *ec*. It may well be that the condition at the white locus in this mutant is repeated at the echinus locus, but no satisfactory test-stocks exist to demonstrate conclusively that homozygous deficiency of the echinus locus is compatible with survival.

3. *The paradox of w^{258-45}* : If it is true that the white locus is confined to band 3C2, then how can the existence of male-lethal white mutants such as w^{258-45} , said to lack band 3C1 but not 3C2, be explained? Two possibilities can be considered. First, in such mutants the white locus may not actually have been deleted, but rather, may have mutated simultaneously with the occurrence of a chromosome break immediately to its left. If this were true, however, w^{258-45} should complement with *sp-w*. As GREEN (1959b) has shown, and we can confirm, w^{258-45} does not complement with *sp-w*; this implies that the white locus is truly deficient in w^{258-45} .

Alternatively, band 3C2 as well as 3C1 may be absent in w^{258-45} . A detailed

FIGURE 4.—The cytology of two X-chromosome deficiencies. **A** and **B**. Salivary chromosome preparations of $w^{ma} N^{63b}/+$ females. (**A** shows an unsynapsed strand.) Note persistence of both 3C1 and 3C2. **C**. $Df(w-ec)^{64d}/+$ female. Note that 3C1 remains in the deficient chromosome, but not 3C2.

cytological analysis of our w^{258-45} stock suggests that band 3C2 is, in fact, missing (Figure 5A, B). It is quite apparent that our stock of w^{258-45} is deficient for considerably more than a single band or two (Figure 5A) and cannot be an unchanged derivative of the original mutant described by SUTTON (in BRIDGES and BREHME 1944). According to JUDD (personal communication), from whom our stock of w^{258-45} was obtained, a second short deficiency just to the left of 3C1 has

FIGURE 5.—The cytology of w^{258-45} . **A.** Salivary chromosome preparation of $w^{258-45}/+$ female. Note deficiency in 3B region. **B.** Unsynapsed strand showing that, in the left end of the 3C region, only band 3C3 remains. (The w^{258-45} chromosome illustrated here has acquired a second short deficiency just to the left of the original w^{258-45} deficiency. See text.)

arisen. This second deficiency can, with difficulty, be separated from w^{258-45} , thereby restoring the original condition. This complication, however, does not affect the critical consideration, which is whether or not w^{258-45} possesses band 3C2. As shown especially well in Figure 5B, which illustrates an unsynapsed strand, only band 3C3 remains. Thus, we interpret w^{258-45} as being deficient for both 3C1 and 3C2. We are supported in this conclusion by EILEEN SUTTON GERSH, who made the original analysis of w^{258-45} and who has also confirmed our interpretation of the euchromatic breakpoint in w^{mJ} (personal communication).

In an effort to substantiate our conclusion that in w^{258-45} the white locus is not contained within band 3C1, we produced a duplication containing band 3C1 but not 3C2. This was done by irradiating $\gamma^+ w^{mJ}$ males, crossing them to γw attached-X females, and recovering a fragment containing, as euchromatic material, only the left end of the chromosome, that is to say, from γ^+ through 3C1. Adding this fragment to γw^{258-45} , we recovered viable γ^+ white-eyed males. The fragment covered the lethality associated with w^{258-45} without affecting the eye color. This result strengthens our principal conclusions that the white locus is confined to band 3C2 and that the absence of 3C2 alone is compatible with survival. An independent locus to the left of 3C2, on the contrary, is essential for

survival. Thus, the white locus is surrounded by two lethal loci, one to its left and one to its right. Male-lethal white mutants, then, must be deficient not only for 3C2, but also for one or the other, or both, of the adjacent lethal loci. Male-viable white mutants may be either point mutations (in which case they will complement with *sp-w*) or deficiencies for part or all of 3C2, but not including the surrounding lethal loci (in which case they will not complement with *sp-w*).

DISCUSSION

1. *Position effects in inversions:* The evidence presented above argues strongly for the localization of the white gene in salivary chromosome band 3C2. Considering the limitations of salivary chromosome cytology, however, we cannot absolutely deny the possibility that the white locus occupies a position between bands 3C1 and 3C2 or, with less likelihood, between bands 3C2 and 3C3. On the other hand, we feel that the evidence is sufficient to eliminate the possibility that the white locus extends over two or more salivary chromosome bands. All of our evidence is consistent with the hypothesis that the white locus is, in fact, confined to band 3C2.

Additional mutants affecting the white locus, which may appear in the future, could permit more rigorous testing of our hypothesis. Of particular value would be other white-mottled inversions similar to w^{m4} and w^{mj} . If a third white-mottled inversion should give duplication and deficiency crossover products with both w^{m4} and w^{mj} , then a more precise localization of the white locus should be possible. Unfortunately, white-mottled inversions are exceedingly rare, despite the fact that white-mottled translocations are relatively common.

We believe that the reason for this is related to a greater spread of position effect from the point of chromosome breakage in translocations than is commonly encountered in inversions. In order to express a variegated phenotype, the white locus need not be very close to the breakpoint when translocated to a heterochromatic region of another chromosome. In T(1;4) w^{258-21} , for example, the X chromosome is broken at 3E5; yet, variegation for white is observed (BRIDGES and BREHME 1944). By contrast, in inversions it appears that the white locus must lie immediately adjacent to heterochromatin in order to express variegation. In w^{m4} , where band 3C2 has been placed next to proximal heterochromatin, typical white variegation is observed, but variegation for roughest (presumably in band 3C4) is not observed. In w^{mj} , where heterochromatin has been inverted so as to lie distally immediately next to band 3C2 (which remains in its normal location), again typical white variegation is observed. Here, too, there is no evident variegation for roughest; whereas, in rst^g , which has band 3C4 immediately adjacent to heterochromatin, there is strong variegation for roughest, but none for white. These facts suggest that, unlike the situation in translocations, there is little, if any, spread of position effect from the point of breakage in X chromosome inversions.

As shown by w^{mj} , it is not necessary for the locus itself to be inverted and in contact with proximal heterochromatin in order for variegation to occur; variegation can be expressed equally well when heterochromatin is inverted and brought into immediate contact with the locus. In this sense, then, there is no

polarization of position effect; the effect is the same whether the heterochromatin adjoins the locus on its left side or on its right. The spread of position effect in X chromosome inversions may be so slight that if but a single independent locus (or band) intervenes between the junction with heterochromatin and the locus in question, variegation will not normally be observed. We are, in fact, inclined to predict that when further white-mottled inversions are discovered and analyzed, their left breakpoints will be found to coincide with that of either w^{m4} or w^{mJ} .

It might be pointed out that the white variegation exhibited by w^{mJ} is not related to the special circumstance whereby w^{mJ} has become translocated to Chromosome 4. The lethal recombinant chromosome, $w^{mJL-rst^{sR}}$, which in effect is the w^{mJ} chromosome no longer translocated but borne on an X-chromosome centromere, still expresses white variegation when made heterozygous with white. (See Figure 2.) Thus in w^{mJ} , white variegation is a function of the influence at the left breakpoint alone; it is not a consequence of the fact that w^{mJR} contains no X chromosome heterochromatin.

2. *Male-viable deficiencies*: In a few instances, homozygous deficiency of specific genes can be tolerated by the organism (MULLER 1935; PANSIN 1938). The present studies indicate that the white locus is a true member of the group of genes whose presence in at least one dose is not required for survival. Taken at its face value, this implies that the white locus controls no vital function and is thus dispensable. By contrast, loci such as those we postulate to exist just to the right and to the left of the white locus must control vital functions so that their presence in at least one dose is essential for survival.

In the case of white, however, its presumed non-essentiality may be related to the fact that it appears to possess some homology with the zeste locus. As shown by the studies of Judd (1961b) and GREEN (1963), zeste on occasion will synapse with white, thereby permitting asymmetrical exchange. If this synaptic affinity between zeste and white reflects genetic homology, then the loss of the white locus might be compensated for by the presence of the zeste locus. If so, it would not be fair to conclude from the viability of homozygous deficient white flies that the white locus possesses no vital function. It may well be that the fly could not tolerate the simultaneous loss of both the zeste and white loci. In the absence of deficiencies for the zeste locus alone, however, this possibility cannot be tested.

We wish to acknowledge our indebtedness to Miss ULLA-BRITT JONSSON for the excellent salivary chromosome preparations, and to Miss BARBARA ALLEN for assistance with the photomicrography.

SUMMARY

Following an extensive cytogenetic analysis of several aberrant X chromosomes containing breaks at or near the white locus, we have concluded that the white locus is contained within salivary chromosome band 3C2. Deficiency for this band in the male does not give rise to a lethal effect; rather, males lacking only 3C2 are viable, fertile, and white-eyed. The survival of flies homozygous deficient for the white locus may be correlated with the apparent homology

between white and zeste. On either side of the white locus are loci whose presence is essential for survival. Mutants that are described as male-lethal white deficiencies lack, in reality, not only band 3C2, but also adjacent bands as well.

Analysis of a new white-mottled inversion, w^{mJ} , indicates that typical white variegation can be expressed when heterochromatin is inverted so as to lie immediately adjacent to the white locus. It is not necessary for the expression of white variegation that the white locus be inverted so as to lie immediately adjacent to proximal heterochromatin, as in the case of w^{m4} . The scarcity of white-mottled inversions, as compared with white-mottled translocations, can be explained by a sharply restricted spread of position effect from the point of chromosome breakage in X-chromosome inversions.

LITERATURE CITED

- BRIDGES, C. B., 1938 A revised map of the salivary gland X-chromosome of *Drosophila melanogaster*. J. Heredity **29**: 11-13.
- BRIDGES, C. B., and K. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552**.
- EMMENS, C. W., 1937 Salivary gland cytology of the roughest³ inversion and reinversion, and roughest². J. Genet. **34**: 191-202.
- GREEN, M. M., 1959a Non-homologous pairing and crossing over in *Drosophila melanogaster*. Genetics **44**: 1243-1256. — 1959b Putative non-reciprocal crossing over in *Drosophila melanogaster*. Z. Vererb. **90**: 375-384. — 1963 Unequal crossing over and the genetical organization of the white locus of *Drosophila melanogaster*. Z. Vererb. **94**: 200-214.
- JUDD, B. H., 1961a Formation of duplication-deficiency products by asymmetrical exchange within a complex locus of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. **47**: 545-550. — 1961b Analysis of products from regularly occurring asymmetrical exchange in *Drosophila melanogaster*. Genetics **46**: 1687-1697. — 1964 The structure of intra-locus duplication and deficiency chromosomes produced by recombination in *Drosophila melanogaster*, with evidence for polarized pairing. Genetics **49**: 253-265.
- LEFEVRE, G., JR., 1952 Report of new mutants. Drosophila Inform. Serv. **26**: 66.
- LEWIS, E. B., 1952 The pseudoallelism of white and apricot in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. **38**: 953-961.
- LINDSLEY, D. L., 1954 On the position of the right breakpoint of $In(1)w^{m4}$ and $In(1)rst^8$. Drosophila Inform. Serv. **28**: 130.
- MULLER, H. J., 1935 A viable two-gene deficiency phenotypically resembling the corresponding hypomorphic mutations. J. Heredity **26**: 469-478.
- MULLER, H. J., and A. A. PROKOFYEVA, 1935 The individual gene in relation to the chromosome and the chromosome. Proc. Natl. Acad. Sci. U. S. **21**: 16-26.
- PANSHIN, I. B., 1938 A viable homozygous deficiency in *Drosophila melanogaster*. Nature **142**: 837. — 1941 Cytogenetic analysis of the homology of genes in reversed linear repeats. Comp. Rend. (Doklady) Acad. Sci. URSS **30**: 57-60.
- RATTY, F. J., 1954 Gene action and position effects in duplications in *Drosophila melanogaster*. Genetics **39**: 513-528.