STUDIES OF REARRANGEMENTS INVOLVING HETEROCHROMATIN IN DROSOPHILA MELANOGASTER

I. A PROOF OF VARIEGATED-TYPE POSITION EFFECT AT THE WHITE LOCUS

II. A STUDY OF THE HETEROCHROMATIN OF CHROMOSOME IV

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ABSTRACT

The X-IV translocation, w^{258-21}, is shown to contain the wild-type allele, w^+, at the white locus. This w^+ has been replaced with a mutant gene, w, and a comparison of $R(w^+)/w$ with $R(w)/w^+$ shows the former to give a variegated white phenotype while the latter is completely wild-type. It is concluded that the white variegation is due to an instability in the action of w^+ when it is located in the rearranged chromosome.

Cold temperature enhances variegation particularly when applied during the embryonic stages of development. A less sensitive period is found to exist during the pupal stages. These facts indicate the white gene is active during more than a single period of development.

Twelve duplication-deficiency types have been obtained by combining the left and right parts of four X-IV translocations. Tests for survival of these combinations in the haplo-IV condition give somewhat contradictory results. These results are discussed and a possible order for the fourth chromosome translocation breaks is given.

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I. A PROOF OF VARIEGATED-TYPE POSITION EFFECT AT THE WHITE LOCUS

INTRODUCTION

The variegated-type position effect in Drosophila is generally the result of a euchromatic-heterochromatic rearrangement of the chromosomes. Such a change in the normal position of a gene with regard to its neighbors often causes it to become unstable in its action. This instability results in somatic variegation to give a mosaic phenotype. A large number of genes of Drosophila are affected in this manner when placed in close proximity with heterochromatin, but only a few are suitable for studying this phenomenon. Considerable evidence has been collected in proof of the variegated-type position effect but this is all indirect with the exception of two cases, one by Dubinin and Sidorov (1935) for the hairy locus, and the other by Panshin (1935) for the gene curled.

Dubinin and Sidorov studied a translocation between the third and fourth chromosomes which showed a variable change in the expression of the hairy gene located in the left arm of chromosome III. Cytological examination of the rearrangement showed one break near hairy and the other in the heterochromatic region near the fourth chromosome centromere. Flies heterozygous for the mutant allele hairy, (h), and the translocation, R(h^+), (R = any euchromatic-heterochromatic rearrangement) showed a few microchetes on the scutellum varying in number between 1 and 11, the average being

4.77. This compares to "several dozens" for the homozygous hairy genotype and none for wild-type. These investigators replaced the original hairy locus of the translocated chromosome with a mutant hairy gene, (h), by obtaining a crossover between hairy and the translocation break point. Utilizing this newly constructed R(h) chromosome they introduced a new normal hairy allele, (h_2^+), from a structurally normal chromosome into the translocation by a similar crossover. The newly introduced gene (h_2^+) now exhibited a variable hairy genotype similar to the allele in the original translocated chromosome. A comparison of the combinations R(h^+)/h, R(h_2^+)/h, and R(h)/h^+ showed that even though these combinations are structurally identical, only the first two show the variable hairy phenotype, while the third is completely wild-type. This proves that a position effect is exhibited by this translocation because the h^+ gene changes its action only when it is located in the rearranged chromosome.

The experiments carried out by Panshin on another translocation involving the third and fourth chromosomes produced similar results. Heterozygotes for the translocation and the mutant curled (cu) in the third chromosome showed a variable curled phenotype ranging from total absence of wing curvature, as in normal individuals, to a marked degree of curvature similar to the homozygous curled phenotype. Panshin reported that 0.8% crossing over was observed between cu and the translocation break point. The curled locus of the original translocation was introduced into a chromosome of normal structure by recovering such a crossover; this allele

proved to be the dominant wild-type under these conditions. A mutant cu gene was inserted in the translocation by crossing over and this chromosome was used to introduce a new cu^+ gene from a normal chromosome. Panshin then showed by a classification of the degree of curled exhibited by the offspring of the new $R(cu^+)/cu$ and the original $R(cu^+)/cu$ that both exhibit similar variability for the curled phenotype. Again in this case cu^+ shows instability in its action only when it is in the rearranged chromosome.

Some of the best examples of variegation in Drosophila are those involving the somatic instability of the white locus (Muller, 1930; Schultz, 1936; Panshin, 1938; Demerec, 1940; and others). The compound eyes, each with about 750 ommatidia, offer excellent material for studying mottling because a change in the color of only a few facets can be easily detected. It is not necessary to rely on the variability of the phenotype within a population for detection or classification of variegation.

Cytological studies of white-mottled types by Schultz (1936), Sacharov (1936), and particularly by Demerec (1941), offer strong indirect evidence for position effect in these cases. These investigations have shown that in every case of white variegation the locus of white has been moved to close proximity with one of the heterochromatic regions. Griffen and Stone (1940a) concluded, however, that the presence of heterochromatin is not necessary to induce mottling, and that this association between w^+ and heterochromatin in the white-variegated types could be explained by a

decided tendency for breakage to occur in the region of white (3C of Bridges, 1938, chromosome map) and proximal heterochromatin. They were led to this conclusion by their observations of the X-4 translocation, w^{m5}. This rearrangement places w^{+} near the centromere of the fourth chromosome and shows a distinct mottling for white. Cytological examination indicated to these authors that the fourth chromosome was entirely euchromatic (Griffen and Stone, 1940b). In their study of the partial and complete reversals of w^{m5} after exposure to x-rays they found that further rearrangements which placed the white locus again in euchromatin did not always result in a return to wild-type.

The work of several investigators (Bridges, 1935; Schultz, 1936; Bauer, Demerec and Kaufmann, 1938; and others) raises serious objection to the entirely euchromatic nature of the fourth chromosome. Their observations of the fourth chromosome's staining and pairing characteristics have indicated the presence of proximal and terminal heterochromatin. Assuming there are heterochromatic regions in chromosome IV, the inability of Griffen and Stone to obtain complete reversal of white mottling in all cases where 3C was returned to euchromatin may be explained, as pointed out by Kaufmann (1942), by assuming that some heterochromatin was transferred to euchromatin along with the white region in these cases. Further evidence implicating heterochromatin in cases of white variegation was presented by Demerec (1941) who showed that wholly euchromatic rearrangements involving the white region were either not effective in causing a

change in the action of w^+ or were associated with a stable change of the white gene. Also pertinent to this discussion are data presented by Kaufmann (1946) on the distribution of X chromosome breaks and the relative recombination frequency of the breaks for given regions. He showed that though the region of the white locus (3C) and the heterochromatic region of X are among those sections which give a relatively high frequency of breakage, there is no preferential recombination between these regions, and that rearrangements with a break near the white locus more often involve euchromatic than heterochromatic sectors.

Additional indirect evidence for position effect is found in the analysis of products obtained following x-ray treatment of rearrangements giving variegation. Panshin (1938) studied the X-4 translocation, w^{m11}, in which the white locus has been transferred to the heterochromatin of the short arm of chromosome IV (4R). Cytological examination of 25 complete or partial reversals of white showed that eight were complete reversals which were the result of transferring the white locus to euchromatin or in one case to the distal heterochromatin of X. Of the 13 incomplete reversals, 6 were rearrangements which replaced heterochromatin of 4R with euchromatin while 7 were of the same general type as the eight true reversals, presumably with some heterochromatin transferred to euchromatin with region 3C. Panshin also found that extreme white-variegated types derived from x-raying w^{m11} were the result of placing additional heterochromatin near the white

locus. These results show that the action of the w^+ gene is dependent upon the proximity and quantity of heterochromatin and that a return to normal is effected by removal of the heterochromatic region. The data obtained by Griffen and Stone (1940a) mentioned above may also be interpreted in this manner. Schultz (1941) points out, however, that the effect of superimposing another rearrangement on these mottled types makes interpretation of the results very difficult. The effect of the new rearrangement by itself is not known and it is difficult to dissociate position effect and gene interaction in these cases.

The "spreading effect" exhibited by euchromatic-heterochromatic rearrangements may also be explained in terms of position effect. This phenomenon is characterized by a tendency for the heterochromatin to affect the activity of a number of genes of the euchromatic sector placed next to it. A good example of this is the X-4 translocation w^{258-21} studied by Schultz (1941). In females heterozygous for the translocation and the mutants w and spl, he found that the facets of the eyes showing changes at the white locus always showed split, that is, the facets were roughened and disarranged, while some facets showing split may be wild-type in color. Since the locus for split is nearer the heterochromatin than is the white locus it appears that the effect exerted by the heterochromatin is spreading along the chromosome and modifying the action of the genes nearest it most extensively. Demerec and Slizynska (1937) found the same relationship to exist for white

and roughest in the eyes of homozygotes or hemizygotes of w^{258-18}. Demerec (1940) found that in the inversion N^{264-52} the effect of the heterochromatin spread through some 50 bands of the salivary chromosome.

Despite the considerable amount of indirect evidence indicating a position effect in the case of white-variegating types, it was felt that direct proof is needed to clarify further the position effect phenomenon.

EXPERIMENTAL

The X-4 translocation w^{258-21} was selected for this study. Fig. 1b shows that the region to the left of 3E5 in the X chromosome has been moved to the heterochromatic region near the centromere of chromosome IV. The X chromosome genes diminutive (dm), facet (fa), split (spl), and white (w), show variegation in flies heterozygous for the translocation and the mutant gene, particularly when grown at temperatures below 25°C. This translocation also shows a dominant Notch phenotype (N) that is probably due to variegation. The translocation is lethal in the hemizygote.

Replacement of w^{+} in w^{258-21}

The break in the X chromosome of w^{258-21} is far enough to the right of the white locus that crossing over should occur between white and the break point, yet flies are easily classified for varie-

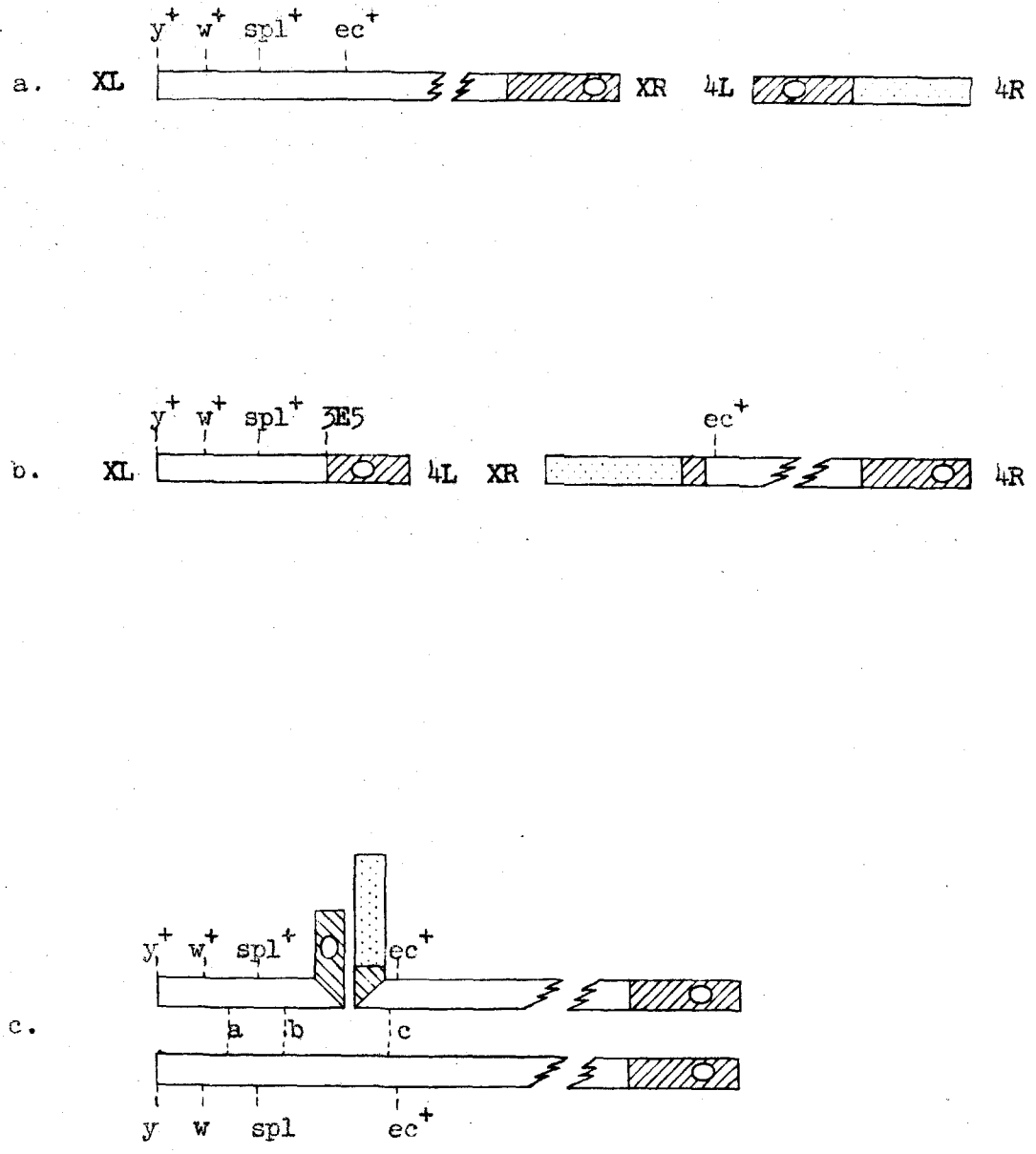

Figure 1. a. Diagrammatic representation of the wild-type X and fourth chromosomes, showing the positions for the genes yellow (y), white (w), split (spl) and echinus (ec). b. The X-4 translocation, w^{258-21}, (diagrammatic). c. Diagrammatic representation of the pairing relationship between the X-4 translocation and a normal X chromosome.

Legend: unshaded = euchromatin of X; shaded = heterochromatin of X or 4; stippled = euchromatin of 4; open circles = centromeres of X or 4.

gation of white due to the "spreading effect" in this rearrangement. An experiment was set up in an attempt to replace the white locus of this rearranged chromosome with a known mutant white gene, (w). The markers used were yellow (y), split (spl), and singed (sn^3). Females were made heterozygous for the translocation and the X chromosome carrying y w spl sn^3. These females were then made heterozygous for rearrangements in the second and third chromosomes, since rearrangements in the two major autosomes are known to increase greatly the amount of crossing over in the X (Steinberg, 1936). Two Curly inversions (Bridges and Brehme, 1944) were used in the second chromosome. The left arm is marked by the dominant Curly, (Cy), while the right arm carries speck (sp^2). Since sp^2 is recessive, the presence of the right arm inversion is not always insured. For the third chromosome the complex rearrangement Ubx^{130} (Ubx = Ultrabithorax) was used (Lewis, 1952). This rearrangement is associated with a dominant bithorax-like change and is lethal in the homozygous condition.

Females of the above constitution (i.e., w^{258-21}/y w spl sn^3; Cy sp^2/+; Ubx^{130}/+) were mated to y w spl sn^3 males in half-pint milk bottles on standard culture medium. The offspring were examined for crossing over between w and spl. Females with the phenotype y w spl^+ would be crossovers which placed the tip of the normal X chromosome through the locus of w into the translocation. Such an event is represented by a crossover at point a in Fig. 1c. Two females of this phenotype were recovered from approximately 22,000 female off-

spring examined; one proved to be sterile while the other gave y w and y w spl daughters and y w spl sons when mated to her y w spl sibs. The y w daughters were of the type desired and these were mated individually to males carrying the X chromosome balancer "Complete" (contains $In(1)sc^{8}$, dl-49, y^{31d} w^{a} lz^{s} B, see Bridges and Brehme, 1944). Using the slight Notch phenotype as a marker for the translocation, daughters were again mated singly to "Complete" males and a true breeding stock was established. Females from this stock were outcrossed to y males and slides were made of the salivary glands from the y larvae. Cytological examination of the salivary gland chromosomes showed the translocation to be present with the breaks being at essentially the same points as described for the original w^{258-21} (Bridges and Brehme, 1944). The cytology was checked by Dr. E. B. Lewis.

The complementary crossover class of the one just described could also be detected from the experimental set-up outlined above. This crossover would transfer the distal end of the translocated X chromosome to a chromosome of normal structure. Two males of this class were recovered, both of which had normal wild-type body and eye color (y^{+} w^{+}). One carried spl, while the other was spl^{+}; both were sn^{3}. These males represent crossovers between white and the break point of the rearrangement, one event having occurred between white and split (point a in Fig. 1c) to give y^{+} w^{+} spl sn^{3}, the other between split and the break point (point b in Fig. 1c) giving y^{+} w^{+} spl^{+} sn^{3}. The conclusion must be reached that the white and

split genes of the original translocated chromosome are the wild-type alleles, w^+ and spl^+. It is entirely possible, but highly unlikely, that the male showing only sn could be the result of contamination since a sn^3 stock is present in the laboratory. The other male showing the markers spl and sn^3 can be accounted for most simply by crossing over as outlined above since a stock carrying these markers is not present. These two males were mated individually to y f attached-X females. A stock was established from each. In both cases the male offspring in these stocks continued to show completely wild-type eye color.

Crossing over in the tip of the translocated X chromosome is reduced to essentially zero. It has been possible to obtain the four cases of crossing over described above only through the presence of the second and third chromosome rearrangements. Steinberg (1936) has shown that the Cy inversions greatly increase crossing over in the y end of the X chromosome. Lewis (1952) has used the Ubx^{130} rearrangement to advantage in obtaining an increased crossing over in the distal end of the X chromosome. Even with the aid of these autosomal rearrangements the crossing over in this region of the X chromosome of $w^{258\text{-}21}$ heterozygotes is extremely small, being approximately 0.0091% (3/33,000 $\pm$) in the region between w and spl.

Experiments were set up to determine whether the mottling observed in heterozygotes of constitution $R(w^+)/w$ is also exhibited in the $R(w)/w^+$ flies. Preliminary observations indicated that the former shows variegation for white while the latter is completely

normal. It is known that the presence of a Y chromosome in the $R(w^+)/w$ heterozygotes will reduce the somatic variegation of the genes in the translocation to such an extent that the phenotype is wild-type (Gowen and Gay, 1933, 1934). Gowen and Gay demonstrated that temperature also greatly modifies the expression of variegation in white-mottled types. They point out that high temperature suppresses mottling while low temperature enhances it. The effect of the Y chromosome, however, dominates that of the temperature in the sense that types showing mottling at 18°C. appear wild-type at this temperature if an extra Y chromosome is present. For these reasons care was taken to control these modifying factors while comparison of the $R(w^+)/w$ and $R(w)/w^+$ was done.

Single $R(w^+)$/"Complete" and R(w)/"Complete" females were out-crossed to males of constitution In(1)dl-49, y Hw m^2 g^4 [an X chromosome inversion stock, delta-49, containing the markers yellow (y), Hairy wing (Hw), miniature (m^2) and garnet (g^4) (see Bridges and Brehme, 1944)]. The offspring from all females showing secondary non-disjunction were discarded thus eliminating any XXY females. The $R(w^+)$/In(1)dl-49, y Hw m^2 g^4 daughters from the above mating were crossed with males carrying y w ec f or to males of type y w spl sn^3. The R(w)/In(1)dl-49, y Hw m^2 g^4 females were crossed to males carrying w^+ spl sn^3, or w^+ spl^+ sn^3 both types of which were described above as being derived by crossing over from the original translocated chromosome. 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 of the culture was not known until all the results had been tabulated. It was found that $R(w^+)/w$ showed variegation for w; 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, but 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 white-apricot, w^a; white-buff, w^{bf}; white-cherry, w^{ch}; white-coral, w^{co}; white-eosin, w^e; white-honey, w^h; white-ivory, w^i; white-satsuma, w^{sat}; and white 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.

Lewis (1952) has demonstrated that the genes w and w^a are pseudoallelic in nature rather than true alleles as was formerly supposed. In view of this finding, an effort was made to place w^a in the translocation so that a study of the position effect in $R(w^a)/w$ could be made. The experimental set-up was similar to that described previously. Females heterozygous for the newly derived $w^{258\text{-}21}$ y w and a normal X chromosome with the markers

y^2 w^a spl ec, and carrying one or both of the rearrangements SM1 (Lewis and Mislove, 1953) or In(2LR)102, ds^W in the second chromosomes and either Ubx^{130} or 3LP Sb 3RC or both in the third chromosomes were mated singly to y w ec f males. The offspring were examined for females showing y^2 w^a ec^+; this phenotype should represent a crossover between the white locus and the break point of the translocation. This set-up is superior to the previous one in that the crossover may be detected if it occurs at any place in the region between w and the break point whereas in the former experiment only those crossovers occurring between w and spl could be detected.

Four y^2 w^a ec^+ females were found among the offspring of approximately 250 fertile cultures, but none was the desired crossover type. Two of these were quite infertile and subsequent progeny tests showed them to be triploids. The triploid condition (i.e., w^{258-21} y w/y^2 w^a spl ec/y w ec f for the X chromosomes) gives a phenotype similar to the desired crossover. The other two females proved to be the result of crossing over to the left of ec and to the right of the translocation break point (point c in Fig. 1c). This was shown by their offspring when they were mated to y w ec f males. The majority of the sons were y^2 w^a spl or y w ec f; some were recombinants of these two parental classes. The daughters proved to be of the same types, heterozygous for the paternal y w ec f chromosome. An event such as this was not forseen when the experiment was set up to use ec as the significant marker. It was supposed that since ec is located so near the break point a cross-

over between the two is extremely unlikely. Echinus is placed at 3F1-2 by Demerec and Sutton (Demerec et al., 1942) while the break point of w^{258-21} is known to be between 3E5 and 3E6 (Bridges and Brehme, 1944) in the X chromosome. According to Bridges' (1938) revised map of the X chromosome there are only three bands separating these two points. It is entirely possible that such a crossover could occur, however, since pairing of the X chromosomes should be good at this point. Schultz (1941) reports that in the salivary gland chromosomes of w^{258-21} the 3F1-2 bands are almost always closely paired. The existence of these two females can best be explained by such a crossover, therefore it may be concluded that such events did occur.

Lethal Effect of w^{258-21}

In the experiments designed to replace certain parts of the translocated X chromosome of w^{258-21} it was noted that all parts of this X chromosome except the spl-ec region could be recovered in a chromosome of normal structure by crossing over. All of these products lived as males, therefore, the recessive lethal effect of w^{258-21} must lie in the spl-ec region, that is, very close to the break point. It is felt that this lethality might be the result of variegation for a gene or genes in this region. The presence of an extra Y chromosome is known to reduce variegation in w^{258-21} very markedly therefore one or more extra Y chromosomes might make it possible to recover w^{258-21} as a male. Single w^{258-21}/"Complete"

females were crossed to In(X^{c2}) f males known to be XYY. An extra Y chromosome was known to be present in some of the females also. The matroclinous females that appeared from this cross were mated to their brothers in an effort to build up an excess number of Y chromosomes. From 11 such cultures two males appeared that apparently carried the translocated X chromosome and probably one or more extra Y chromosomes. This could not be confirmed since both males were sterile. The eyes of these males were slightly smaller than normal and the facets were irregularly arranged; there was no detectable variegation for white. Several thoracic bristles were missing (dorsocentrals and scutellars), and the microchetes were sparse and disarranged. It is entirely possible that the extra heterochromatin of the Y chromosome is acting to reduce variegation to such an extent that these males live.

The Effect of Low Temperatures on White-Variegation

During the course of the experiments to determine the effect of low temperatures on white-variegation an attempt was made to ascertain the temperature sensitive period for w^{258-21}. Females of genotype w^{258-21}/In(1)dl-49, y Hw m^2 g^4 were mated to y w ec f males and allowed to lay eggs for a 24 hour period. These cultures were then exposed to either 19°C. or 14°C. for varying lengths of time during the development of the flies. The offspring from these cultures were classified subjectively into four groups, 0, 1, 2, 3 depending on the amount of variegation exhibited in the eyes. Class

0 represents those flies which showed no detectable mottling in either eye. Class 1 represents those that showed a change in only a few facets. Class 2 consists of those which showed patches of mutant tissue extending up to 1/4 of the area of the eyes. Class 3 represents those which exhibited mottled patches extending over more than 1/4 of the area of the eyes. The two eyes of the fly were averaged for this classification. Table 1 contains the pertinent data obtained from these experiments.

The time of treatment is listed in days after the eggs were deposited, 0 being the day they were laid. From the Chi-square values given in Table 1, it can be seen that the groups treated at 19°C. and 14°C. for the same period of development are not significantly different from one another. The groups treated in the 0-3 day period do differ significantly from those treated during the 4th-9th day and both of these groups differ significantly from the group that was grown throughout development at 25°C. The most sensitive period lies in the very early stages, that is, 0-3 days after the eggs were laid. This is indicated by the fact that groups a and b differ markedly from both groups c and d and e. There seems to be another period of lesser sensitivity that lies somewhere in the later larval and pupal stages since groups c and d are significantly different from group e. The pattern of variegation is quite significant in the flies treated at 0-3 days compared to those treated at 4-9 days. Early treatment results in predominantly large light patches while later treatment gives a

Table 1

The effect of low temperatures on white-variegation in w^{258-21}

Group	Temperature	Time of treatment	Class				
			0	1	2	3	Total
a	19°C.	0-3rd day	5	30	42	26	103
b	14°C.	0-3rd day	0	16	31	25	72
c	19°C.	4-9th day	58	17	5	5	85
d	14°C.	4-9th day	32	16	5	1	54
e	25°C.	0-9th day	112	20	5	0	137

Group	X^{2}*	n	p
a vs. b	3.39	1	.07
c vs. d	1.69	1	.20
a vs. c	90.54	2	$<<0.01$
b vs. d	71.92	2	$<<0.01$
a vs. e	153.36	2	$<<0.01$
c vs. e	7.20	1	<0.01

* X^{2} classes were combined whenever the expected numbers fell below five.

more speckled appearance to the eye with numerous small mutant patches.

Chen (1948) studied the temperature sensitive period for

white variegation in w^{258-18} and w^{m5}. Both of these X-4 translocations are very similar to w^{258-21}. He found the sensitive period for both of these rearrangements to be during the late larval stage and throughout the pupal stages. This is comparable to the 4-9 day treatment described in Table 1. Chen failed, however, to consider the very early egg stages which in the present study were found to be considerably the more sensitive of the two. Schultz (1941) points out that the data of Gowen and Gay also indicate that the early embryonic stages may be the period most sensitive to low temperatures.

DISCUSSION

The results from these experiments show conclusively that the somatic instability of the white gene in the translocation w^{258-21} is due to position effect. The w^{+} gene of the original translocation shows a variegated phenotype in the eyes of females heterozygous for w^{258-21} and w. When this w^{+} allele is transferred to an X chromosome of normal structure this instability is lost and the gene functions as the standard dominant 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 that greatly enhance variegation in $R(w^{+})/w$ flies. Such a result is not in agreement with the interpretation of the position effect phenomenon expressed by Ephrussi and Sutton (1944). By this interpretation

variegation is the result of the upset of pairing relationships of chromosome regions adjacent to the breaks of chromosome aberrations. A change in stress upon the gene might be expected to change its activity. On this hypothesis variegation should occur in both $R(w^+)/w$ and $R(w)/w^+$ since the pairing relationships are the same in each type. This was not found to be the case.

The conclusion indicated by these facts is that instability is conferred only on the allele in the structurally abnormal chromosome while the allele in the normal chromosome retains its usual activity. The mutant allele w would not be expected to show any instability in its action 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 for white must, therefore, be a result of inactivation of the w^+ gene or its product, and this inactivation occurs only when w^+ is in the abnormal chromosome.

Instability in the action of the spl^+ gene may also be inferred from these experiments. Variegation for split is seen in $w^{258\text{-}21}$ heterozygotes yet it is shown that the rearranged chromosome carries the normal allele for split. The dominant variegation for Notch is also consistent with the assumption that inactivation of the gene or its product results from the close association of heterochromatin with the Notch locus. A stable Notch phenotype is with few exceptions the result of a deficiency for the 3C7 band of Bridges' (1938) chromosome map and is always associated with a

recessive lethal effect. No deficiency for 3C7 is seen in w^{258-21} and furthermore the Notch phenotype becomes more extreme in flies cultured below 25°C. This indicates that Notch is due to a position effect. The lethal effect in w^{258-21} is also probably due to variegation for the Notch or some other lethal locus located close to the break point since addition of extra Y chromosomes suppresses the lethality.

The significance of the experiments on the temperature sensitive period in w^{258-21} is twofold. First it is shown that $R(w)/w^{+}$ does not show variegation even under the conditions most likely to produce it. Secondly, some insight into the time of action of the white gene can be gained. The fact that temperature is effective during two periods of the development of the individual is evidence for the concept of "repetitive action" of genes introduced by Stern and Schaeffer (1943). This concept is that a gene has a single action which is repeated at different places or times in development as opposed to the concept of primary gene action as a single event in development from which one or more further events may follow. This "repetitive action" may be due to either continuous or recurrent activity of the gene.

In the case under study it seems reasonable that temperature is affecting the action of the white gene <u>per se</u> rather than a product of the gene. The temperature treatment can be visualized as affecting the rate of chemical reactions but this hardly explains why the temperature is effective at two different periods, unless

the gene itself is actually active at these times. It is not sufficient to imagine the white gene as being active at only one specific period with the final phenotypic expression depending on the fate of its product. It seems likely then that the white gene possesses a potentiality for repeated action throughout development, this being expressed during the embryonic and the pupal stages.

SUMMARY

1. The variegation for white in the translocation $w^{258\text{-}21}$ is shown to depend on the position of the w^+ gene. The white locus from the translocation, when placed in an X chromosome of normal structure, behaves as the wild-type allele, w^+. The mutant w was placed in the translocated X chromosome and a comparison of the types $R(w^+)/w$ and $R(w)/w^+$ shows that the former gives a variegated white phenotype while the latter is completely wild-type. The possibility that variegation is due to the structural heterozygosity in the translocation heterozygotes is excluded by these facts and it is concluded that white variegation is due to instability in the action of the w^+ gene when it is in the rearranged chromosome.

2. The translocation is shown to carry the wild-type allele for split. It is inferred that the variegation for split exhibited by the translocation is due to position effect. The variable Notch

phenotype of w^{258-21} is also consistent with the assumption that instability in the action of the genes in the proximity of the translocation break point is the result of their change in position.

3. The recessive lethal effect of w^{258-21} is shown to be suppressed by addition of an extra Y chromosome(s). It is concluded that the lethality is probably due to variegation at the Notch locus or some other lethal locus near the translocation break point.

4. White variegation is shown to be most sensitive to low temperature during the embryonic stages. The existence of another less sensitive period during the pupal stages is indicated. These findings are discussed in terms of "repetitive action" for the white gene.

II. A STUDY OF THE HETEROCHROMATIN OF CHROMOSOME IV

INTRODUCTION

Heterochromatin assumes a major role in variegated-type position effect in Drosophila. A considerable amount of work has been done on variegating types but not much is known about specific heterochromatic regions with regard to their ability to influence the action of juxtaposed euchromatic genes. Demerec (1941) has presented evidence that the proximal heterochromatic regions of the X chromosome and the autosomes are effective in producing variegation of w^+ when this gene is moved to close proximity with them. Demerec has also shown that differences exist within a given heterochromatic region in its potentiality for inducing variegation. Though close association with heterochromatin seems necessary to produce instability of a euchromatic gene such an association is not always sufficient to induce mottling. Demerec points out that heterochromatin is more efficient in this respect if it is attached to the spindle fibre region. Additional information about the nature of specific heterochromatic regions certainly seems necessary if an understanding of variegated-type position effect is to be reached.

Chromosomal rearrangements with a break in the proximal heterochromatin of IVR very often exhibit a position effect at the cubitus interruptus locus (ci). This phenomenon known as the

"Dubinin effect" (Dubinin and Sidorov, 1934a, 1934b) is characterized by a weakened dominance of ci^+ when tested against the mutant ci. The R(ci^+) types have been the object of study by a number of workers (see Lewis, 1950, for review). The evidence presented by Khwostova (1939) from salivary gland chromosome analysis of x-ray induced R(ci^+) cases showed that nearly all were chromosomal rearrangements involving euchromatin and heterochromatin. The only exceptions found were rearrangements involving fourth chromosome heterochromatin and the distal heterochromatic regions of the X or Y chromosomes, or proximal heterochromatin that had been removed from the centromere region by further rearrangement. In general any rearrangement which removes the ci^+ gene from the proximal heterochromatin of the fourth chromosome to any region distal to a centromere will cause a weakened dominance of ci^+. The ci^+ gene normally occupies a position in or near the proximal heterochromatin of chromosome IV and seems to be dependent upon this proximity for its normal action. This is the reverse of the relationship existing between normally euchromatic genes and heterochromatin. The present study was made in an attempt to learn more about the nature of the proximal region of chromosome IV.

EXPERIMENTAL

The stocks employed were the X-IV translocations w^{258-21},

N^{264-12}, w^{258-18}, and w^{m5} (Bridges and Brehme, 1944). These rearrangements have very similar, though not identical, points of breakage in the X and fourth chromosomes. Figure 2 shows that all four translocations have one break in the white-Notch region of the X chromosome and the other in the proximal heterochromatin of IVR. The breaks in the X chromosomes have been located both genetically and cytologically (Bridges and Brehme, 1944) while the breaks in the fourth chromosome are known only to be in or near section 101 of Bridges' (1935) salivary gland chromosome map.

w^{258-21}

This rearrangement has been described earlier as having a break in X between 3E5 and 3E6 and in IV following 101F. The rearrangement is male lethal and shows mottling for diminutive (dm), Notch (N), facet (fa), split (spl), roughest (rst) and white (w), but not for echinus (ec) or bifid (bi) in the X chromosome. The translocation shows a weakened dominance of ci^+ when tested over ci.

N^{264-12}

The break in the X chromosome is between 3C5-6 and 3C7, and in the fourth chromosome about 101F. According to Demerec (Bridges and Brehme, 1944), this translocation shows mottling for dm, fa, spl, rst and w, but not for prune (pn), kurz (kz), or ec in heterozygous females (see Fig. 2). The Notch characteristic is not always distinct but it is not clear whether this is due to

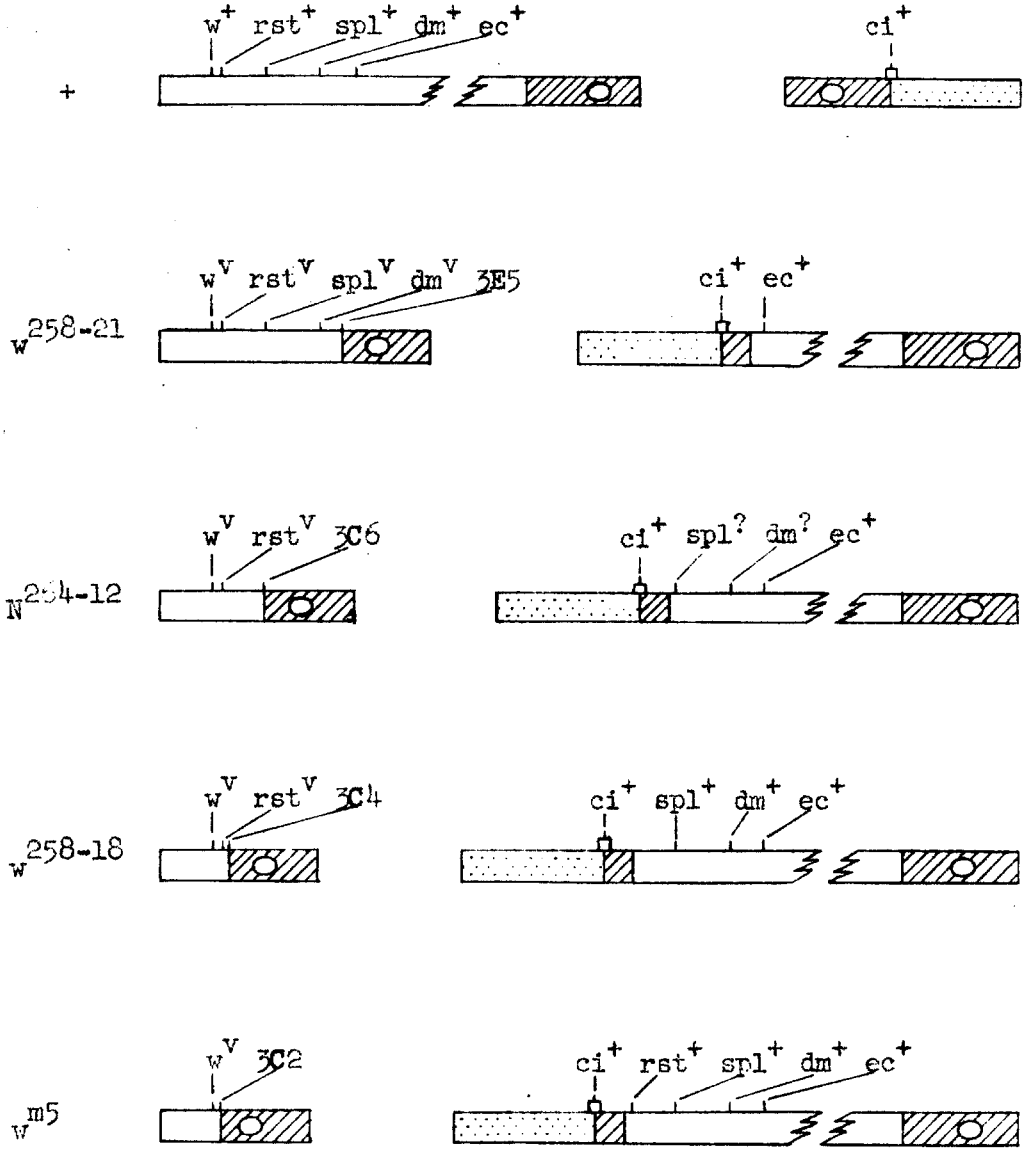

Figure 2. Diagrammatic representation of a normal X and fourth chromosome, and four X-4 translocations. Relative positions and variegation for the genes white (w), roughest (rst), split (spl), diminutive (dm), echinus (ec), and cubitus interruptus (ci) are given. Positions of the X chromosome breaks are given. Legend: shaded = X or 4 heterochromatin; unshaded = X euchromatin; stippled = 4 euchromatin; open circles = X or 4 centromeres.

variegation. Notch is usually associated with a deficiency for 3C7, however, salivary chromosome analysis of this rearrangement by Sutton (Bridges and Brehme, 1944) showed 3C7 to be present on the proximal side of the break point in X. If variegation for Notch is occurring, this is an exceptional case since as a rule the X chromosome genes on the proximal side of the break in this type of translocation do not show variegation. N^{264-12} shows the "Dubinin effect" when tested against ci.

w^{258-18}

This well-studied white-mottled arose from an x-rayed y male and is viable in the male and homozygous female. The break in X is after 3C4 and is in section 101 of the fourth chromosome. Mottling occurs for rst and w but not for pn, fa or dm. There is a weakened dominance of ci^+ when tested over ci.

w^{m5}

This translocation lives as a male and as a homozygous female and shows mottling for w but not for rst or fa. The break in X is between 3C2 and 3C3 and between 101F1 and 101F2 in the fourth chromosome (Griffen and Stone, 1938). Bolen (1931) reported from genetic tests that the break in IVR is between bent (bt) and eyeless (ey), but reinvestigation by Sturtevant (1951) places the break to the left of both bt and grooveless (gv1). The rearrangement shows the "Dubinin effect" for ci^+.

The proximal region of IVR is very difficult to study cytologically and for this reason the relative positions of the breaks of these translocations are unknown. Location by genetic tests has been done only roughly since the number of known genes in this region is small and since crossover tests are next to impossible due to the smallness of chromosome IV and the reduced crossing over in the region of the break point.

The method used in attacking this problem was first suggested by Muller (1930) and employed later by Dobzhansky and Schultz (1934) and by Patterson et al. (1937). It consists of combining the parts of two different translocations to obtain duplications and deficiencies. For example, it is clear that unless the translocation breaks are at identical points in the fourth chromosomes, the right part of one when combined with the left part of any of the others, will result in a short duplication or a short deficiency, depending on the relative positions of the break points. This same relationship holds for the X chromosomes as well.

Since it is for all practical purposes impossible to obtain markers in the rearranged fourth chromosome the combinations were recovered using X chromosome markers and a dominant marker, either dominant cubitus interruptus, (ci^D), or dominant eyeless, (ey^D), in the free fourth chromosome.

There are twelve different combinations obtainable using the four translocation stocks, for example the distal portion of the X chromosome of w^{258-21} can be combined with the proximal part

of the X chromosome from N^{264-12}. Such a derivative will be designated in terms of the X chromosome regions as $w^{258-21}L.N^{264-12}R$; from this it is understood that the tip of the X chromosome attached at 3E5 to the centromere region of IV (101F) from w^{258-21} is combined with the proximal portion of the X chromosome of N^{264-12} which has the distal part of the fourth chromosome attached to it (101F-3C7). This combination results in a duplication of the X chromosome for the region 3C7 to 3E5 and in either a duplication or deficiency for a part of region 101F in the fourth chromosome. In order to determine the condition existing in the fourth chromosome of these combinations, an attempt was made to obtain them in the haplo-IV condition on the assumption that the duplications would live and the deficiencies would die under these circumstances. The relative positions of the breaks in the heterochromatic region of IV could be established in this manner.

To make such a scheme effective it is necessary that the normal fourth chromosome present in these stocks be marked, since as Beadle (1933) has pointed out, the translocation of the fourth chromosome causes frequent non-disjunction of the free IV which would establish an equilibrium condition of two normal fourth chromosomes plus the one involved in the translocation. The presence of an extra fourth chromosome would of course make the results obtained from these experiments very difficult to interpret, so the four translocation stocks were cleared of extra fourth chromosomes and those remaining were marked with ci^{D} or ey^{D}. These dominant

markers are lethal in the homozygous condition so non-disjunction of the free fourth chromosome marked in this manner would result in an inviable product.

The method employed to establish this stable diplo-IV condition was to cross females heterozygous for the X-IV translocation and the In(1)dl-49, y Hw m^2 g^4 chromosome to males of constitution T(3;4)c Ubx/ci^D (Bridges and Brehme, 1944). This translocation involving the third and fourth chromosomes has the dominant marker Ubx in IIIR which is broken near curled and attached to the proximal part of the fourth chromosome; the other fourth chromosome of this stock carries ci^D. The III-IV translocation causes a high rate of non-disjunction for the free IV so that from this cross females of constitution T(1;4)/+; T(3;4)c Ubx/ci^D can be obtained. These females are triplo-IV; two of the fourth chromosomes are involved in translocations and the free one carries ci^D. It is also possible to get tetra-IV progeny from this cross through non-disjunction in both parents but it was assumed that such individuals would not survive; if, however, these are viable and fertile the presence of the unmarked IV would become evident in subsequent generations. These triplo-IV females were then crossed to In(1)dl-49, y Hw m^2 g^4; ey^D/+ males and by non-disjunction of the fourth chromosomes in the female parent, offspring of the constitution T(1;4)/In(1)dl-49, y Hw m^2 g^4; ey^D were obtained. In the case of w^{258-18} and w^{m5} which are male viable, males carrying the X-IV translocation and a fourth chromo-

some marked with ey^D were also recovered; these when mated to their sisters of the above constitution gave balanced stocks with a marked free fourth chromosome. The other two translocations, w^{258-21} and N^{264-12}, are male lethal so these had to be crossed to In(1)dl-49, y Hw m^2 g^4; ci^D/+ males and selected for the marked fourth chromosome every generation since males carrying the In(1)dl-49, y Hw m^2 g^4 chromosome with both ey^D and ci^D in the fourth chromosomes proved to be sterile. Later In(1)dl-49, y Hw v^0 f; ci^D/ey^D males were obtained which were fertile and these were used to balance these stocks.

Since only w^{258-18} and w^{m5} are male viable, special procedures had to be followed in obtaining some of the twelve combinations. These methods along with the phenotypic characteristics of each combination are listed under the appropriate section below.

$\underline{w^{258-18}{}_L.w^{m5}{}_R}$

This combination results in a duplication of the bands 3C3 and 3C4 in the X chromosome. The locus of rst is known to be located in 3C4 (Bridges and Brehme, 1944), therefore, no mottling for rst appears in flies of this type even though w^{258-18} shows rst variegation. White variegation does occur as is expected. The w^{258-18} stock carries yellow (y) in the tip of X; therefore, from the cross of w^{m5}/ci^D females by y w^{258-18}/ci^D males it is possible to recover a y male that shows mottling for white but not for roughest. These did appear and they were mated to females

heterozygous for In(1)dl-49, y Hw m^2 g^4 and carrying ci^D and ey^D in the fourth chromosomes. The yellow daughters of genotype y $w^{258\text{-}18}L.w^{m5}R$/In(1)dl-49, y Hw m^2 g^4; ci^D were selected and mated to y $w^{258\text{-}18}L.w^{m5}R/ci^D$ males to establish a stock.

$w^{m5}L.w^{258\text{-}18}R$

This is the complementary type of the one above, being deficient in X for the bands 3C3 and 3C4. It does live as a male and as a homozygous female despite the deficiency, and these show a rather extreme rst phenotype with eye facets irregular in size and shape and the microchaetae sparse and irregular. Variegation occurs for w.

This type was obtained by mating w^{m5}/In(1)dl-49, y Hw m^2 g^4; ci^D females by y $w^{258\text{-}18}/ey^D$ males. Any y^+ Hw females that appear must be either the desired $w^{m5}L.w^{258\text{-}18}R$/In(1)dl-49, y Hw m^2 g^4 or matroclinous females due to primary non-disjunction of the X chromosomes. Primary non-disjunction occurs very rarely but to guard against such an event each y^+ Hw female was mated singly to In(1)dl-49, y Hw m^2 g^4; ci^D/+ males. A stock was established from a culture that gave offspring of the expected phenotype.

$w^{258\text{-}21}L.w^{258\text{-}18}R$

This combination results in a duplication of 3C5 to 3E6. Variegation occurs for w and rst to give striking eroded white patches in the eyes; only a few flies show this in cultures grown

at 25°C. A thickening of the wing veins particularly at the base of the wing near the cross veins is evident in males and homozygous females.

To obtain this type, females of the genotype $w^{258\text{-}21}$/In(1) dl-49, y Hw m^2 g^4; ey^D were mated to y $w^{258\text{-}18}$/ci^D males. Any y^+ Hw females that appear should be of the desired type unless primary non-disjunction has occurred. The females with this phenotype were mated singly to In(1)dl-49 y Hw m^2 g^4; ey^D/+ males and a culture that gave $w^{258\text{-}21}{}_L.w^{258\text{-}18}{}_R/ey^D$ males was selected for the marked fourth chromosome and inbred to give a balanced stock.

$w^{258\text{-}18}{}_L.w^{258\text{-}21}{}_R$

This is the complement of the preceding type and therefore is a deficiency for 3C5 to 3E6. This does not survive as a male and heterozygous females are Notch with reduced viability and fertility. Mottling for white occurs in females heterozygous for w.

$w^{258\text{-}21}$ cannot be used as a male in obtaining this type and since the type itself will live only as a heterozygous female, the proximal part of $w^{258\text{-}21}$ ($w^{258\text{-}21}{}_R$) must be passed from mother to daughter by non-disjunction. Primary non-disjunction occurs very rarely so a Y chromosome was introduced into $w^{258\text{-}21}$/In(1) dl-49, y Hw m^2 g^4; ci^D females to take advantage of secondary non-disjunction which occurs in XXY females. The presence of a Y chromosome was detected by using the third chromosome rearrangement associated with a variegating Stubble mutant [Sb^V; salivary gland

chromosome analysis by Lewis (unpublished) showed the breaks to be 88/89B/41A]. An extra Y chromosome modifies the Stubble phenotype such that all the bristles are Sb while XX females show some non-Stubble bristles. The marker garnet (g^2) was placed in $w^{258\text{-}21}{}_R$ by crossing over so that the non-disjunctional product could be recognized. The cross then was XXY females of genotype $w^{258\text{-}21}$ g^2/In(1)dl-49, y Hw m^2 g^4; Sb^v; ey^D by y $w^{258\text{-}18}$/ey^D males. Secondary non-disjunction for $w^{258\text{-}21}$ g^2R will give some phenotypically y Hw g^2 females that are Notch. These may or may not have an extra Y chromosome so the Sb^v was to be used to select against them; it was found however that these females had such low viability and fertility that Sb^v was lost from the stock before it could be completely cleared of extra Y chromosomes.

$w^{258\text{-}21}{}_L.w^{m5}{}_R$

This type shows white variegation but this appears only rarely in cultures grown at 25°C. In males or homozygous females there is a thickening of the wing veins similar to $w^{258\text{-}21}{}_L.w^{258\text{-}18}{}_R$, but there is no variegation for rst as is found in this type. $w^{258\text{-}21}{}_L.w^{m5}R$ is the longest of the X chromosome duplications, extending from 3C2 to 3E5.

The cross that gave this type was $w^{258\text{-}21}$/In(1)dl-49, y Hw m^2 g^4; ey^D females by y $w^{258\text{-}18}{}_L.w^{m5}R/ci^D$ males. These males were used so that the y mutant could be used as a marker for the tip of X. Any y^+ Hw females should be of the desired type unless

primary non-disjunction occurred. These females were mated singly to In(1)dl-49, y Hw m^2 g^4; ey^D/+ males and the cultures giving $w^{258\text{-}21}L.w^{m5}R$ males were selected for the marked free fourth chromosome and inbred to establish a stock.

$\underline{w^{m5}L.w^{258\text{-}21}R}$.

The deficiency created in the X chromosome by combining these two parts extends from 3C2 to 3E5 and causes this combination to be male lethal and gives an extreme Notch phenotype in heterozygous females. Mottling for white occurs when tested over w.

Secondary non-disjunction was again employed to obtain this stock. Females of constitution y $w^{258\text{-}18}L.w^{258\text{-}21}$ g^2R/In(1) dl-49, y Hw m^2 g^4; ey^D from a stock known to carry extra Y chromosomes were mated to w^{m5}/ci^D males. Using y as a marker for the tip of the X chromosome and g^2 as a marker for the proximal part, females showing y^+ Hw g^2 should be of the phenotype desired. These did appear and a stock was established. This type as yet has not been freed of extra Y chromosomes. Due to the decreased viability of this combination, a sufficient number of these females have not yet been used in the haplo-IV tests to make the results significant.

$\underline{w^{258\text{-}21}L.N^{264\text{-}12}R}$

Even though both of the stocks from which this combination is derived are male lethal, this does live as a male and as a homozygous female. This type is duplicated for the section of X from

3C7 to 3E5 and shows variegation for white in heterozygous females, though this is infrequent in cultures grown at 25°C. Males of this type are very rarely Notch but heterozygous and homozygous females frequently are.

This combination was derived by crossing N^{264-12} females to $w^{258-21}{}_L.w^{258-18}{}_R$ males. The origin of these parental males has been described above. N^{264-12} was marked with g^2 in the right part of the X chromosome so that the y^+ g^2 males that arose are of the correct genotype. These were crossed to In(1)dl-49, y Hw m^2 g^4; ci^D/ey^D females to establish a stock. It was found that this combination with a free fourth chromosome marked with ey^D or ci^D is poorly viable and almost sterile, therefore, these could not be cleared entirely of unmarked fourth chromosomes. For this reason, the data from the haplo-IV test outlined later may have little significance for this combination.

$N^{264-12}{}_L.w^{258-21}{}_R$

This stock contains a short deficiency extending from 3C7 to 3E5 in the X chromosome; therefore, it is male lethal and shows a rather extreme Notch phenotype in heterozygous females. Only a few females have been recovered and a balanced stock has not yet been established. It is known that variegation for white occurs when tested against w, but the haplo-IV tests are incomplete.

Females of this type were obtained by crossing y $w^{258-18}{}_L$.

$w^{258\text{-}21}$ g^2/In(1)dl-49, y Hw m^2 g^4; ey^D females with an extra Y chromosome to $N^{264\text{-}12}L.w^{258\text{-}18}R/ci^D$ males. Recovery of this type is dependent upon non-disjunction for $w^{258\text{-}21}$ g^2R and the In(1) dl-49, y Hw m^2 g^4 chromosomes so by using y Hw and g^2 as markers in the X chromosomes the desired type can be recognized as y^+ Hw g^2.

$N^{264\text{-}12}L.w^{258\text{-}18}R$

This combination results in a duplication in the X chromosome extending from 3C5 to 3C6 inclusive. This is a very short duplication and no phenotypic effect can be attributed to it; males and homozygous females show extreme variegation for rst and w as expected.

This stock was obtained by mating $N^{264\text{-}12}$ g^4/In(1)dl-49, y Hw m^2 g^4; ci^D females to y $w^{258\text{-}18}/ci^D$ males. Females that appear y^+ Hw g^+ are of the correct genotype; males that are y^+ g^+ also appear but these were not used since they probably lack a Y chromosome and are sterile. The females were mated to In(1)dl-49, y Hw m^2 g^4; ey^D/+ males and from this XY males appeared and were used to establish a homozygous stock.

$w^{258\text{-}18}L.N^{264\text{-}12}R$

This, being the complement of the type above, is deficient in the X chromosome for bands 3C5 and 3C6. It is Notch in phenotype in the heterozygous female, and is male lethal. Variegation occurs

for w^{+} when tested against w.

This combination can be obtained without having to depend on secondary non-disjunction. This is possible since $N^{264\text{-}12}R$ can be transmitted through the male by using the combination $w^{258\text{-}21}L.N^{264\text{-}12}\ g^{4}R$. Males of this type crossed to y $w^{258\text{-}18}$/In(1)dl-49, y Hw m^{2} g^{4}; ci^{D} females will give some females that are phenotypically y Hw g^{4} and Notch. These mated to In(1)dl-49, y Hw m^{2} g^{4}; ci^{D}/+ males established the desired stock. Again females of this combination with a dominantly marked fourth chromosome proved to be almost sterile and as yet the stock has not been completely freed of unmarked IVs.

$N^{264\text{-}12}L.w^{m5}R$

Males and homozygous females of this type show extreme variegation for w but not for rst; this is expected since there is a short duplication in X for the bands 3C3 to 3C6. $N^{264\text{-}12}L$ ordinarily shows variegation for rst but in this combination the rst locus (3C4) is covered by the duplication.

This combination was obtained by crossing $N^{264\text{-}12}\ g^{4}$/ In(1)dl-49, y Hw m^{2} g^{4}; ci^{D} females by y $w^{258\text{-}18}L.w^{m5}R/ci^{D}$ males. Females from this cross that appear y^{+} Hw g^{+} are of the desired type; males of this phenotype also appeared but they were not used since they probably lacked a Y chromosome. To establish a stock the above females were mated to In(1)dl-49, y Hw m^{2} g^{4}; ey^{D}/+ males; the offspring were selected for the marked free fourth chromosome

and inbred.

w^{m5} L.N $^{264-12}$R

A short deficiency in the X chromosome extending from 3C3 to 3C6 results from combining these two translocation parts. This combination is male lethal and gives a Notch phenotype in heterozygous females. Variegation appears for white when tested against w.

The combination was obtained by crossing XXY females of genotype y w^{258-18}L.N$^{264-12}$ g^4R/In(1)dl-49, y Hw m^2 g^4; ci^D to w^{m5}/ci^D males. Secondary non-disjunction will give some y^+ Hw g^4 females in the F_1. It is possible to obtain the desired type by crossing w^{m5} females to w^{258-21}L.N$^{264-12}$ g^4R males but there is no marker in either of the X chromosome tips, therefore, it is felt that the former cross is the more reliable. This stock has not as yet been cleared of unmarked free fourth chromosomes so selection is necessary.

Haplo-IV Tests

The duplications and deficiencies obtained from combining the left and right parts of these translocations are well defined for the X chromosomes involved, but the conditions existing with regard to the proximal region of the fourth chromosomes are unclear. It might be expected that fourth chromosome deficiencies resulting from these various combinations would be lethal in the homozygous

or hemizygous conditions. With this in mind an attempt was made to test each of the twelve combinations under one or both of these conditions. Five of the twelve combinations are not male viable; in order to test all the combinations for the haplo-IV state under the same conditions, females heterozygous for each of the translocation combinations and the In(1)dl-49 chromosome were crossed to (1) haplo-IV males; (2) T(3;4)c Ubx/ci^{D} males.

The origin of the haplo-IV stock which was used for the type 1 crosses lies in a single haplo-IV male obtained from crossing T(3;4)c Ubx/ci^{D} males to wild-type females. The T(3;4)c causes non-disjunction of the fourth chromosomes and flies that show neither Ubx nor ci^{D} and are Minute (M; see Bridges and Brehme, 1944) are assumed to be haplo-IV. A single M male was crossed to wild-type females and a haplo-IV stock was established. Such a stock cannot be balanced so selection for Minute has to be done every generation. A maximum of 50% of the offspring from the type 1 cross should be haplo-IV.

The type 2 cross takes immediate advantage of the non-disjunction for the fourth chromosomes that occurs in T(3;4)c. Beadle (1933) reports that the non-translocated fourth chromosome is distributed approximately at random, that is, independently of its translocated homologue in diplo-IV females heterozygous for T(3;4)c. A maximum of 25% of the offspring from crosses of type 2 could then be expected to be haplo-IV. Despite the lower percentage of haplo-IV offspring, type 2 offers a distinct advantage over type 1

since the markers Ubx and ci^D can be used to recognize the haplo-IV products while in type 1 only the Minute character can be used for classification, and it is quite possible that other Minutes could arise in the stock which would mimic the haplo-IV Minute.

The results obtained from crosses of type 1 and type 2 are recorded in Table 2. The findings from both type 1 and type 2 crosses were the same so these have been combined for clarity.

The most striking aspect of these data is the marked sexual dimorphism for survival in the haplo-IV condition. Without exception the four original translocations and the twelve combinations derived from them fail to live as heterozygous females hemizygous for the translocated fourth chromosome. However, five of seven combinations that are male viable do survive as males hemizygous for the translocated fourth chromosome. The two exceptions are $w^{m5}L.w^{258\text{-}18}R$ and $w^{258\text{-}21}L.N^{264\text{-}12}R$. It should be noted that only one haplo-IV $w^{m5}L.w^{258\text{-}18}R$ male was observed and subsequent tests designed to confirm this finding gave negative results. In the case of $w^{258\text{-}21}L.N^{264\text{-}12}R$, the stock has not been completely cleared of extra free fourth chromosomes which may account for the negative results here.

As is pointed out above, females heterozygous for the translocation and the In(1)dl-49, y Hw m^2 g^4 X chromosome were used for the haplo-IV tests, therefore, the haplo-IV In(1)dl-49, y Hw m^2 g^4 males and heterozygous females were used as controls in the type 1 crosses. Negative tests, that is failure to recover the

Table 2

Survival of translocation combinations under various fourth chromosome conditions

Genotype	Survival as Haplo-IV ♂	Survival as Haplo-IV ♀	Survival as Homozygous Diplo-IV Females	Survival over $Df(4)M_4$ as Males and/or Females
$w^{258\text{-}18}L.w^{m5}R$	+	-	+	+
$w^{m5}L.w^{258\text{-}18}R$	-*	-	+	+
$w^{258\text{-}21}L.w^{258\text{-}18}R$	+	-	+	+
$w^{258\text{-}18}L.w^{258\text{-}21}R$	0	-	0	+
$w^{258\text{-}21}L.w^{m5}R$	+	-	+	+
$w^{m5}L.w^{258\text{-}21}R$	0	***	0	***
$w^{258\text{-}21}L.N^{264\text{-}12}R$	-**	-**	-**	+**
$N^{264\text{-}12}L.w^{258\text{-}21}R$	0	***	0	***
$N^{264\text{-}12}L.w^{258\text{-}18}R$	+	-	+	+
$w^{258\text{-}18}L.N^{264\text{-}12}R$	0	-**	0	+
$N^{264\text{-}12}L.w^{m5}R$	+	-	+	+
$w^{m5}L.N^{264\text{-}12}R$	0	-**	0	+
$w^{258\text{-}21}$	0	-	0	+
$N^{264\text{-}12}$	0	-	0	+
$w^{258\text{-}18}$	+	-	+	+
w^{m5}	+	-	+	+
T(3;4)86D	+	+	+	***

\+ denotes survival.
\- denotes lethal.
0 denotes type not recoverable because of X chromosome deficiency.
* A single haplo-IV male of this type was observed.
** Stock not completely free of unmarked fourth chromosomes.
*** Type not tested.

translocation in the haplo-IV state, were considered significant only when 20 or more haplo-IV flies were observed in these control classes. Such a control could not be used for the type 2 crosses since all of the free fourth chromosomes carry a dominant marker that is lethal in hemizygotes.

The question arises as to whether this sexual dimorphism is common to all translocations involving the fourth chromosome. Beadle (1933) apparently had no trouble obtaining haplo-IV females heterozygous for T(3;4)c. As a further test females homozygous for T(3;4)86D, bx^{34e} [a III-IV translocation with breaks in 86D and 101F (Lewis, unpublished); it carries the markers bithorax, bx^{34e}, and ebony, e] were mated to haplo-IV males. As is shown in Table 2, both males and females heterozygous for this translocation survive as haplo-IV. These results indicate that the sexual dimorphism is limited to the X-IV translocations and the derived combinations.

A further complication is found among those types that survive as homozygous females. In these females both of the fourth chromosomes are involved in the translocations and as was pointed out earlier, those combinations which result in fourth chromosome deficiencies might be expected to be lethal in this homozygous state. Table 2 shows that with one exception the homozygous diplo-IV females do survive. This exception again is $w^{258\text{-}21}L.N^{264\text{-}12}R$.

Other Tests on the Fourth Chromosomes

Each of the translocations and 10 of the derived combinations

were tested against the fourth chromosome deficiency Minute-4, M_4. Cytological examination of this deficiency shows a loss of about 10 bands near the base of 4R (Bridges and Brehme, 1944). It includes the loci: abdomen rotatum, ar; cubitus interruptus, ci; grooveless, gvl; and Scutenick, Scn. It was hoped that some of the combinations would prove to be lethal opposite this deficiency and in this way possibly clarify the results from the haplo-IV tests. It was found however that the four original translocations and all of the combinations tested were viable opposite M_4. Two combinations, $w^{m5}L.w^{258\text{-}21}R$ and $N^{264\text{-}12}L.w^{258\text{-}21}R$ were not tested.

Each of the combinations was also crossed to the mutant abdomen rotatum, ar. This is the most proximal gene known in 4R and it was hoped that some of the translocation combinations might result in a fourth chromosome deficiency that would "uncover" this gene. All the combinations gave negative results.

As was pointed out earlier, each of the four original stocks show the "Dubinin effect," that is, a decrease in the dominance of ci^+ when tested against ci. Heterozygous females from each of the combinations were crossed to y w ec f; ci ey^R males to test for the "Dubinin effect" and for white variegation. All of the combinations showed a decrease in dominance of ci^+ and all showed white variegation.

DISCUSSION

The fact that none of the translocations lives as heterozygous females in the haplo-IV condition makes the analysis of the fourth chromosome breaks rather complex. A number of factors must be considered. The most obvious of these is the effect of the X chromosome deficiencies. It is quite possible that the Notch characteristic and the haplo-IV condition act in a cumulative manner such that all of the X chromosome deficiency combinations die as haplo-IV regardless of whether there is a duplication or a deficiency in the fourth chromosome. $Df(1)N^{8}$ which is an X chromosome deficiency extending from just left of 3C1 to just left of 3E1 (Bridges and Brehme, 1944) was used to test this effect. This is a simple X chromosome deficiency with no fourth chromosome involvements so such a stock can be used to measure the effect of the X chromosome deficiency on the survival of haplo-IV flies. It was found that heterozygous N^{8} females do survive as haplo-IV but their viability is about 0.25 that of the non-deficient controls. This is indeed a striking reduction in the viability of such flies, so assuming that all of the X chromosome deficiency combinations die as haplo-IV, an attempt at analysis of the fourth chromosome breaks can be made from the six X chromosome duplication combinations.

Five of the six live as haplo-IV males. The one exception, $w^{258\text{-}21}L.N^{264\text{-}12}R$, also has not been observed to live as a homozygous diplo-IV female. It has been pointed out that this stock has not been

entirely cleared of free fourth chromosomes and that this may be the reason for the seeming discrepancy. There is reason to doubt however that this factor seriously interferes with the haplo-IV tests. Females heterozygous for this combination and In(1)dl-49, y Hw m^2 g^4 and with an unknown number of unmarked free fourth chromosomes were crossed to Df(4)M_4/ey^D males. Any non-Hw offspring from this cross that show neither ey^D nor M_4 must be $w^{258\text{-}21}$L.$N^{264\text{-}12}$R/M_4/+ since M_4/+/+ appears wild-type. Less than 5% (8/164) of the offspring fell in this class, which indicates that very few if any of the parental females had two free fourth chromosomes. It is probable from this that the haplo-IV tests are valid since a large number of these flies have been tested; 86 haplo-IV individuals of the control classes were observed from these tests.

If it can be assumed by this line of reasoning that $w^{258\text{-}21}$L.$N^{264\text{-}12}$R is deficient for part of the fourth chromosome heterochromatin, the relative positions of the breaks in the fourth chromosome may be located on the basis of haplo-IV male survival. The order on this basis is w^{m5}, $w^{258\text{-}18}$, $w^{258\text{-}21}$, $N^{264\text{-}12}$, with w^{m5} having the most proximal break. Objection to this may be raised of course since by this scheme w^{m5}L.$w^{258\text{-}18}$R males should not survive in the haplo-IV state. It was pointed out previously that one male of this type was observed but that subsequent attempts to confirm it failed. This type is very similar to $N^{264\text{-}12}$L.$w^{258\text{-}18}$R since both have about the same pattern for white variegation and both show an extreme rst phenotype. $N^{264\text{-}12}$L.$w^{258\text{-}18}$R does survive as a haplo-IV male and it is quite

possible that the one $w^{m5}{}_{L}.w^{258\text{-}18}{}_{R}$ male was the result of contamination.

Further objection may be raised to this proposed order for the breaks because it involves the assumption that complementary combinations would behave in opposite ways with regard to the haplo-IV condition. It is assumed for example that since $w^{258\text{-}21}{}_{L}.w^{258\text{-}18}{}_{R}$ lives as a haplo-IV male the complement, $w^{258\text{-}18}{}_{L}.w^{258\text{-}21}{}_{R}$, would die as a male in this condition. Since the latter cannot be recovered as a male because of the X chromosome deficiency, this assumption cannot be tested.

Probably the best argument against the validity of this proposed order for the fourth chromosome breaks stems from the fact that the scheme is based entirely on the male classes that survive in the haplo-IV condition. It is certainly true that the effect of the X chromosome rearrangements is not the same in males and heterozygous females. Variegation for the X chromosome genes is in general more extreme in the hemizygous males as opposed to the heterozygous females. Because of this the effect of the X chromosome rearrangements on survival in the haplo-IV condition may not be comparable for the two types. However, females that are homozygous for the translocation are more comparable to the male classes. Table 2 shows only one discrepancy between those classes that survive as haplo-IV males and as homozygous diplo-IV females; this is the combination $w^{m5}{}_{L}.w^{258\text{-}18}{}_{R}$. On the basis of the order of the fourth chromosome breaks proposed above this type should be deficient for

part of the fourth chromosome. The fact that this type lives in the homozygous state indicates that if there is a deficiency it is not lethal in this condition.

In view of the somewhat contradictory data obtained from the haplo-IV tests, it may be equally valid to approach the problem from the assumption that all of the combinations are viable in the haplo-IV state regardless of the condition in the fourth chromosome and that their survival as haplo-IV depends entirely on the condition in the X chromosome. On this basis the six X chromosome duplications should live and the six deficiency combinations should die as fourth chromosome hemizygotes. This, in general, is true but again there is an exception that must be considered. The fact that $w^{258\text{-}21}L.N^{264\text{-}12}R$ does not live as a haplo-IV male nor as a homozygous female may possibly be explained by the fact that a Notch phenotype is exhibited by this stock even though this combination results in an X chromosome duplication for the Notch region. $N^{264\text{-}12}$ shows a Notch phenotype even though the break in the X chromosome is to the right of 3C7. As was pointed out previously, the Notch phenotype may be due to a stable change rather than variegation. $w^{258\text{-}21}$ also shows a variable Notch phenotype, therefore, it is not surprising that $w^{258\text{-}21}L.N^{264\text{-}12}R$ shows a slight Notch even though the Notch locus is included in the duplication created by combining these translocation parts.

Neither of the schemes just considered can account for the fact that none of the combinations lives as haplo-IV heterozygous

females. It is difficult to imagine that the effect is due entirely to the condition of the rearranged X chromosome since the hemizygous male counterparts, where obtainable, do live as haplo-IV. The most obvious factor to consider here is the effect of the Y chromosome. If it is assumed that the heterochromatic Y chromosome can "cover" deficiencies in the heterochromatin of the fourth chromosome all of the combinations would be expected to live as XXY females. Even if survival of the fourth chromosome hemizygotes is dependent on this "covering" of deficiencies by the Y it is hard to understand why some of the combinations that are actually duplications for part of the fourth chromosome do not live as XX females. Experiments to test the effect of a Y chromosome in females have not yet been completed but even if the Y is the determining factor in haplo-IV survival its action is not simply a "covering" of heterochromatic fourth chromosome deficiencies. Clearly further investigation is needed to determine the reason for the marked difference in survival of haplo-IV males compared to haplo-IV females.

The tests using each of the translocation combinations against Df(4)M_4 (Table 2) show that if fourth chromosome deficiencies are created in these combinations they do not overlap the M_4 deficiency or if they do overlap they are not lethal. Furthermore none of the combinations "uncover" the ar locus which is included in the M_4 deficiency and is the most proximal locus known in 4R.

Tests of each of the combinations against w in the X chromosome indicate that the white variegation is dependent on the distal

segment of the X chromosome and is independent of the proximal part with which it has been combined. Quantitative data were not collected on this point but no obvious changes in the white variegated pattern occurred when different XR segments were combined with each of the XL parts. The best example of this is $w^{258-21}L$. The original w^{258-21} shows little variegation for white in heterozygous females cultured at 25°C. This was found to be true for $w^{258-21}L$. regardless of which right part it was combined with.

Similar results were obtained for the scute locus in a much more elegant study by Raffel and Muller (1940). They studied the three X chromosome inversions, sc^{4}, sc^{L8} and sc^{S1} all of which had the left break in essentially the same position just to the right of 1B3-4. The right breaks were at different points in the heterochromatin of X. Combinations of the left and right ends of these inversions were obtained by crossing over. A comparison of the reduction in number of specific bristles and hairs caused by each of the combinations showed that the effect caused by any one of the left ends was consistently the same irrespective of which right end had been combined with it. The bristle patterns caused by the three right ends in combination with each of the left ends were not significantly different or were inconsistent. As is pointed out by Lewis (1950), the variegation for the scute (sc), Hairy wing (Hw) and achaete (ac) genes can account for the bristle patterns exhibited by the left ends of these inversions.

If the relative positions of the breaks in the fourth

chromosome heterochromatin can be established for the translocations used in the present study, a quantitative study of the "Dubinin effect" could be carried out. Panshin (1938) presented evidence that variegation for the white gene is dependent upon the amount of heterochromatin that was placed next to it. This point could be tested for the ci^+ locus using these translocations. By knowing the relative position of each of the fourth chromosome breaks a measure of the amount of heterochromatin transferred along with the ci^+ locus can be obtained. Carefully controlled experiments may give some indication as to whether this has an effect on the ci phenotype. Admittedly this is a difficult project to carry out since the ci effect is hard to measure quantitatively and the experiments would have to be done using isogenic stocks.

SUMMARY

1. The left and right parts of the X-IV translocations $w^{258\text{-}21}$, $N^{264\text{-}12}$, $w^{258\text{-}18}$, w^{m5} were combined to give twelve X and fourth chromosome duplication-deficiency combinations. The methods used for obtaining each combination and the phenotype exhibited by each are described.

2. Analysis of the proximal region of 4R is attempted by testing each of the combinations in the haplo-IV condition. It was found that none of the combinations live as haplo-IV heterozygous females but five of the twelve live as haplo-IV males. On

this basis tentative positions for the fourth chromosome breaks are established.

3. The effect of the X chromosome deficiencies and the Y chromosome on the survival of the combinations in the haplo-IV state is discussed briefly.

4. The variegation for white in each of the combinations seems to be constant for any given X chromosome left end and is not obviously influenced by the particular right end combined with it.

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