SUPPRESSION OF THE FACET-STRAWBERRY POSITION EFFECT IN DROSOPHILA BY LESIONS ADJACENT TO NOTCH

W. J. WELSHONS AND H. J. WELSHONS

Department of Genetics, Iowa State University, Ames, Iowa 50011

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ABSTRACT

The recessive visible rough eye mutant effect of fa^{swb}, a small deletion at the 5′ end of the Notch locus, is suppressed when fa^{swb} is coupled to five different closely linked deficiencies distal to salivary band 3C7. In addition, an inversion with a proximal breakpoint between 3C3 and 3C5 similarly suppresses the mutant effect. The data support the position effect interpretation of fa^{swb}: The small deletion allows functions distal to Notch to interfere with functions at Notch, and when the interference is eliminated, the fa^{swb}-mutant effect disappears.—The fa^{swb} deletion also interacts with another recessive visible rough-eye mutant at Notch called fa^{g}. In the *cis* condition, fa^{swb} fa^{g} double mutants have a mutant-eye phenotype like fa^{g} (similar to the mutant effect of fa^{swb}) and, in addition, express an accessory phenotype (thickened wing veins). Although the mutant-eye effect of fa^{swb} can be suppressed by lesions adjacent to Notch, the accessory phenotype of the coupled mutants is not suppressed. It is suggested that the fa^{swb} deletion has two observable effects: One is a modifiable position effect causing the fa^{swb} rough-eye phenotype; the other is a stable effect exerted upon a 5-kb insertion that is the probable cause of the fa^{g} mutant expression, thus resulting in a wing effect that accompanies the eye effect of fa^{g}.

THE recessive-visible mutant facet-strawberry, fa^{swb} (synonym: swb^{71b}), was induced by X rays and isolated by LEFEVRE and KELLEY (1972), who demonstrated that it was allelic to Notch on the *X* chromosome. In mutant males, the eyes are rough and glossy; hence, the phenotype is similar to that of the facet-glossy (fa^{g}) allele. Recombination experiments demonstrated that fa^{swb} was at the left end of the genetic map and inseparable from N^{55e11} (LINDSLEY and GRELL 1968). Cytologically, we defined fa^{swb} as a very small deficiency, mostly interband, between 3C5,6 and the Notch band 3C7 on the polytene *X* chromosome (WELSHONS and KEPPY 1975; KEPPY and WELSHONS 1977). The deletion of interband material produced a fusion of 3C6 with 7, causing the doublet 3C5,6 to appear to be slightly enlarged, whereas 3C7 seemed to be missing (Figure 1).

We linked fa^{swb} to $Df(1)w^{67k30}$ (w^{67k30}), a recessive lethal deletion for bands 3C2 to 6 to the left of fa^{swb} that lacks white (*w*), roughest (*rst*) and vertical (*vt*) functions, yet retains an intact Notch locus (LEFEVRE and GREEN 1972). Al-

though crossing over between the closely linked deficiencies was rare, we isolated the two deletions in the *cis* condition and discovered that the tandem arrangement caused the suppression of the fa^{swb} mutant. As an explanation, we suggested that fa^{swb} was a position effect caused by the deletion of DNA sequences that allowed genetic activity in an adjacent region to interfere with normal functions at Notch. When the fa^{swb} deletion was in *cis* to w^{67k30}, Notch functions were restored because the interfering activity was absent in the larger deficiency. It followed from this position effect interpretation that other lesions in the region between white (*w*) and Notch (*N*) could be expected to suppress the mutant effect by eliminating these adjacent functions. One could seek them by mutagenizing fa^{swb} chromosomes and screening for reversions of the mutant phenotype or by linking fa^{swb} to already existing lesions in the region adjacent to Notch. In this paper, we report the consequences of linking four deficiencies to fa^{swb} and of inducing an inversion with a breakpoint between *w* and *N* on a fa^{swb} chromosome. In all cases, the fa^{swb} mutant is suppressed.

MATERIALS AND METHODS

The origin, phenotype, genetic position at Notch and the cytology of fa^{swb} have already been described. The rough, glossy eye, mutant condition is less severe in females than in males; hence, fa^{swb}, like *fa*, is not dosage compensated, whereas the expression of fa^{g} is the same in both sexes. When fa^{swb} is coupled to fa^{g} (facet-glossy), the double mutant can be recognized because an accessory phenotype is expressed. The fa^{swb} fa^{g} double mutant has thickened longitudinal veins that broaden into deltas at the junction with the marginal vein. Heterozygotes fa^{swb}/fa^{g} have rough eyes that are not glossy.

Dp(1;2)51b7 represents a small piece of the *X* chromosome equivalent to w^{+} N^{+} dm^{+} inserted into the right arm of chromosome 2 (LEFEVRE 1952). A derivative, *Dp(1;2)76f*, produced by EMS treatment, represents a deletion of the N^{+} to dm^{+} region in *Dp(1;2)51b7*. The derived duplication covers white (*w*), roughest (*rst*) and verticals (*vt*), but it stops short of *N*.

We have used four deficiencies in the *w-N* region of the *X* chromosome. All have been described in an earlier publication by LEFEVRE and GREEN (1972) in their study of genetic duplication in the *w-spl* interval. $Df(1)rst^{2}$ is a male-viable deletion for bands 3C3 to 3C5 that is mutant for *rst* and *vt* (Figure 1). The remaining three are derivatives of a w^{8}, in turn derived from mutable w^{c} by GREEN (1976). They were called *wrv* mutants because each was mutant for *w*, *rst* and *vt* (LEFEVRE and GREEN 1972). Because our cytological definitions differ from the published report, we will consider them in more detail.

Deficiency *wrv-4*, like rst^{2}, is viable in males and expresses the mutant conditions *rst* and *vt* to about the same degree as in rst^{2}; however, unlike rst^{2}, it is also mutant for *w*. It was defined as a deficiency for salivary bands 3C2 to 5 (LEFEVRE and GREEN 1972), but to us it looks like a deletion for 3C2 with 3C1 fused to a remnant of 3 (Figure 1). Such a fusion yields a band at the 3C1 position that is heavier than 3C7, whereas we had noted earlier that 3C1 equals 3C7 (KEPPY and WELSHONS 1980). According to the published report, the remaining bands are 3C1, 3C6 and 7; by our definition, they represent the fusion of 3C1 with 3, 3C5,6 and 7.

Deficiency *wrv-6* is mutant for *w* to *vt*, is lethal in males and, like $Df(1)w^{67k30}$, was defined as a deficiency for bands 3C2 to 6. To us, it looks like a deficiency for bands 3C2 to 5 with 3C1 fused to a remnant of 6 (Figure 1). In the published report, the remaining bands are 3C1 and 3C7 as in w^{67k30}; we expect them to be the fusion product of 3C1 with 6 followed by 3C7. In hemizygous males, the band at 3C1 is heavier than 3C7, whereas in w^{67k30}, 3C1 and 7 are strikingly similar (KEPPY and WELSHONS 1980). The greater density of 3C1 can also be seen in heterozygotes $Df(1)w^{67k30}/Df(1)wrv\text{-}6$. Recombination data to be reported later support the view that some part of 3C6 is present in *wrv-6*.

Deficiency *wrv-7* is also a lethal deletion previously defined as a deficiency for bands 3C2-6 like *wrv-6* and w^{67k30}. In hemizygous males, we could see 3C7 adjacent to a distal band that was heavy

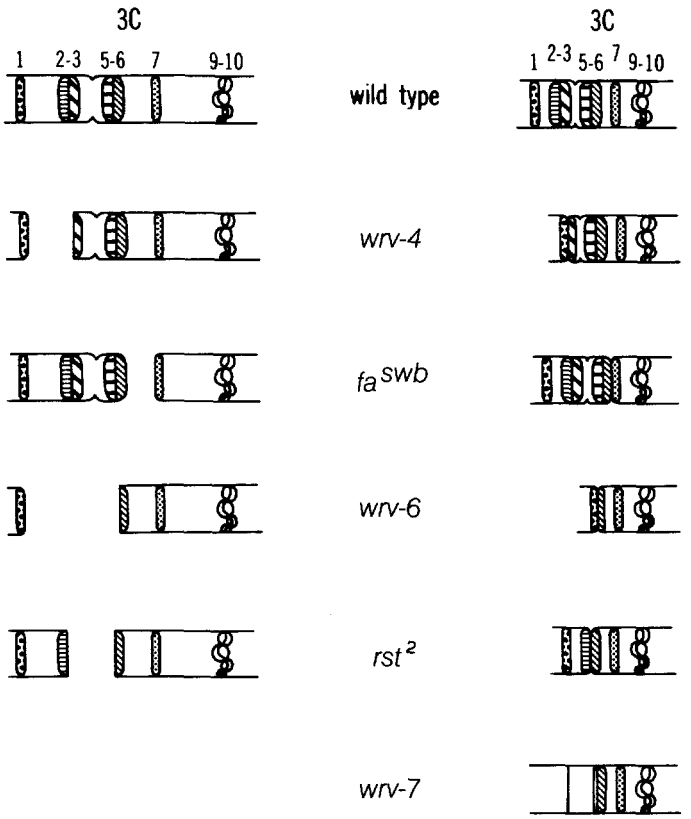

FIGURE 1.—The banding pattern of the polytene-*X* chromosome in the region 3C1 to 10. Salivary bands 4 and 8 are not shown. Expanded, deleted chromosomes are shown on the left, and on the right, the breakpoints are "healed" and the chromosomes are shown in a contracted state. Only the contracted state of *wrv-7* is shown because the breakpoints are not completely defined.

enough to represent a large piece of 3C5,6, and more distally there was a very fine band. In heterozygotes $Df(1)w^{67k30}/Df(1)wrv\text{-}7$, pairing occurred at 3C7, but there was much distortion to the left of 3C7, and we were not able to determine the pairing situation at 3C1 (in w^{67k30}) or at the position of the more distally located fine band (in *wrv-7*). We will not attempt to define breakpoints in *wrv-7*. We can only describe it by saying that distal to 3C7 there is a relatively heavy band followed by a more distally located fine band (Figure 1). Deficiencies w^{67k30} and *wrv-7* are cytologically different, and our recombination data suggest that some part of 3C6 is present.

Obviously, our definitions of these *wrv* aberrations differ from those reported by LEFEVRE and GREEN (1972). Perhaps we have relied more heavily on band size comparisons than other authors have done to arrive at our conclusions (KEPPY and WELSHONS 1980). Perhaps the reactivation of genetic instability in w^8-derived material that ostensively had become stable for a time is a cause of the difference. We already reported the case of N^{66k26}, a "stable" Notch inversion in the region 3C1 to 7. It regained its mutability and produced N^+ by reinversion of the original aberration (WELSHONS and KEPPY 1981). Later, the revertant produced several more *N* mutants. One *N* produced a series of N^+ revertants, and from these it has been possible to implicate highly mutable FB elements (COLLINS and RUBIN 1982; LEVIS, COLLINS and RUBIN 1982) as the probable cause of the instability (GRIMWADE *et al.* 1985). Because the high mutability of the elements could have triggered intervening rounds of mutation, it might be that different investigators have not subjected the same w^8-derived material to microscopic examination.

The description of these *wrv*'s has resulted from the study of many cytological preparations before the experiments were performed and throughout the course of experimentation. No examination of a few microphotographs can resolve the differences of definition, and we have submitted none. Wherever the cytological breakpoints might be at the level of a band or two, only the following three pieces of information are necessary for the demonstrations we wish to make with the *wrv*'s and *rst*^{2}. (1) Each one is an aberration to the left of Notch at 3C7. (2) Each is, to our eyes, cytologically different from the others, and when coupled to the small deficiency *fa*^{swb}, each one changes, but the coupled conditions differ one from another. (3) Microscopic examination throughout the course of experimentation indicated that the material remained cytologically stable.

The *In(1)78b* inversion was induced by ionizing radiation and isolated by D. O. KEPPY. It occurred on a chromosome that already carried *fa*^{swb} and is defined as *In(1)3A2-3;3C3-5*; the *fa*^{swb} mutant effect is suppressed. Normally, we do not inspect the region around zeste (z), but this inversion forced our attention, and we soon discovered that bands 3A3 and 3A4, demonstrated as single bands on Bridges' map (see LINDSLEY and GRELL 1968), are actually double bands, and the doublet at 3A3 might be slightly lighter than the doublet at 3A4. Later, we noted that SORSA and SAURA (1980) had already discovered the double condition of these bands in their electron microscopic study. The doubleness and density differences of 3A3 and 3A4 can be seen in our published phase-contrast figures, although, at that time, we were not aware of their condition (KEPPY and WELSHONS 1980). *In(1)78b* is viable as a male, and we have found no mutant condition associated with it.

Three experiments were performed with each of the four deficiencies; we will use *wrv-6* as an example because the crosses were very similar:

$$\text{A: } \frac{y\ (wrv\text{-}6)\ +\ +\ +}{+\ +\ fa^{swb}\ fa^{g}\ rb} \times w^{a}\ fa^{g}$$

$$\text{B: } \frac{y\ (wrv\text{-}6)\ fa^{swb}\ fa^{g}\ rb}{+\ +\ +\ +\ +} \times w^{a}\ fa^{g}\ rb$$

$$\text{C: } \frac{y\ (wrv\text{-}6)\ fa^{swb}\ +}{+\ +\ +\ rb} \times w^{a}\ fa^{g}\ rb$$

From cross A, we isolated *y* (*wrv-6*) *fa*^{swb} *fa*^{g} *rb* used in cross B from which we obtained *y* (*wrv-6*) *fa*^{swb} used in the final cross. Because *wrv-6* does not survive in males, in the A cross we had to seek the *y* (*wrv-6*) *fa*^{swb} *fa*^{g} *rb* chromosome in heterozygous females that were phenotypically dilute-w^{a} and fa^{g}. Crossing over between *wrv-6* and *fa*^{swb} produces the chromosome we seek (Figure 2), whereas an exchange between *fa*^{swb} and *fa*^{g} (to the right of the deletion in *fa*^{swb}) excludes *fa*^{swb}. Both events yield the dilute-w^{a} fa^{g} phenotype, and the distinction was made by examination of salivary gland preparations from male larvae that carried *Dp(1;2)51b7* to cover the lethality of *wrv-6*. Defining *wrv-6* as a deficiency for 3C2 to 5 with 3C1 fused to 6, consider first the cytology of the *y* (*wrv-6*) + *fa*^{g} *rb* recombinant that we do not seek. Only the *wrv-6* deletion is present; in the region 3C1 to 3C7, only two bands are seen representing the fusion product of 3C1 with 6 followed by 3C7. When the two deletions are coupled to yield *y* (*wrv-6*) *fa*^{swb} *fa*^{g} *rb*, only one band is seen representing 3C1 fused to 6, in turn fused to 3C7 (Figure 2). It is the cytological "disappearance" of 3C7 that is the criterion for distinguishing between *wrv-6* and the coupled condition *wrv-6-fa*^{swb}. With every one of the deficiencies described here, and with *w*^{67k30} previously reported, it is the failure to resolve a unitary 3C7 that signals the presence of the coupled deletions.

In A, the inclusion of *fa*^{g} in the scheme (on the + + *fa*^{swb} *fa*^{g} *rb* chromosome) facilitated genetic screening. Cross B was performed to eliminate the *fa*^{g} *rb* markers and isolate *y* (*wrv-6*) *fa*^{swb} to determine whether or not the *fa*^{swb} phenotype was suppressed. An exchange between *wrv-6* and *fa*^{swb} produces heterozygotes *y* (*wrv-6*)/*w*^{a} *fa*^{g} *rb* with a dilute-w^{a} phenotype. The exchange between *fa*^{swb} and *fa*^{g} yields *y* (*wrv-6*) *fa*^{swb}/*w*^{a} *fa*^{g} *rb*, which will be dilute-w^{a} with rough eyes (like *fa*^{swb}/*fa*^{g}) if *fa*^{swb} is not suppressed and dilute-w^{a} if it is. The two conditions are cytologically distinct.

Cross C was performed to confirm the isolation of the double deletion in cross A by uncoupling the two deficiencies and reisolating *fa*^{swb}. An exchange between *wrv-6* and *fa*^{swb} yields *fa*^{swb}/*w*^{a} *fa*^{g} *rb* females with rough, not-glossy eyes and fa^{swb} males.

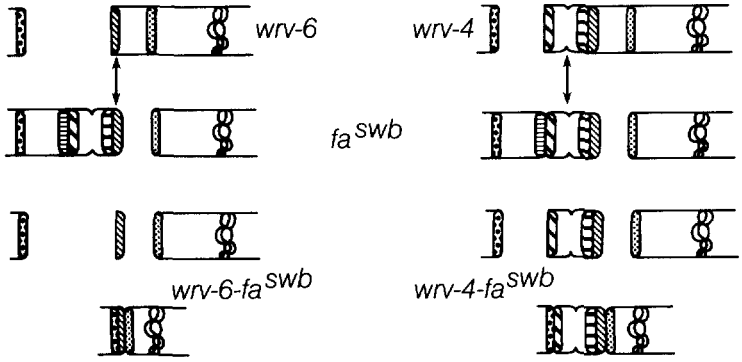

FIGURE 2.—Genetic exchanges between deleted chromosomes. Expanded chromosomes are used to demonstrate the region of exchange between the two deletions (at arrow). The result is seen in expanded form and in the contracted state. The band count between 3C1 and 7 in a *wrv* (or rst^2) deficiency is reduced by one when coupled to fa^{swb} to yield a *wrv* (or rst^2)-fa^{swb} double deletion.

RESULTS

Deficiencies *wrv-6* and *wrv-7* are male lethal, whereas *wrv-4* and rst^2 are male viable. We will describe the crosses using *wrv-6* to be compared with *wrv-7* and *wrv-4* for comparison with rst^2. Examination of male progeny in the non-lethal crosses provided phenotypic data for which there was no counterpart when the recessive lethals were used.

Type A, male-lethal crosses:

$$\frac{y\ (wrv\text{-}6)\ +\ +\ +}{+\ +\ fa^{swb}\ fa^{g}\ rb} \times w^{a}\ fa^{g}$$

We isolated only dilute-w^a fa^g females that could arise by crossing over between *wrv-6* and fa^{swb} or fa^{swb} and fa^g. Reciprocal recombinant products did occur, but we did not collect them because these crosses were contaminated by *Dp(1;2)51b7* that had been used in construction of the heterozygous females. The isolated females were mated to males $y\ w^a\ N^{55e11}$; *Dp(1;2)51b7*/+ so that we could isolate the lethal recombinant chromosome in a male with the *Dp* that would eventually provide cytological material. The mating also allowed us to examine females heterozygous for the recombinant chromosome and N^{55e11} in an attempt to determine whether or not fa^{swb} was suppressed. We had noted earlier (WELSHONS and KEPPY 1975) that N^{55e11}/fa^{swb} females have an extreme Notch-wing phenotype, whereas N^{55e11}/fa^g heterozygotes are not so extremely mutant.

The cross produced 38,200 progeny, but we found only eight females with the appropriate phenotype. None of the recombinant chromosomes showed an enhanced Notch-wing phenotype in heterozygotes with N^{55e11}, suggesting that either all eight lacked fa^{swb} or that fa^{swb} was suppressed. The ensuing cytological analysis demonstrated, however, that three had *wrv-6*-fa^{swb} cytology, whereas five had *wrv-6* cytology (see Figures 1 and 2). It seemed that fa^{swb} was suppressed when coupled to *wrv-6*.

In the male-lethal *wrv-7* cross, we screened 35,300 progeny and found 15

recombinants. Heterozygous with N^{55e11}, none showed an enhanced Notch-wing phenotype, but nine had *wrv-7-fa*^{swb} cytology, whereas six were cytologically *wrv-7*. It seemed that *fa*^{swb} was suppressed when coupled to *wrv-7*.

Type A, male-viable crosses:

$$\frac{y\ (wrv\text{-}4)\ +\ +\ +}{+\ \ +\ \ fa^{swb}\ fa^{g}\ rb} \times w^{a}\ fa^{g}$$

We screened 31,900 progeny. We found 30 dilute-w^a fa^g females, and each was tested by mating to *y w*^{a} N^{55e11};*Dp(1;2)51b7*/+ males. The recombinant chromosomes heterozygous with N^{55e11} did not show enhancement of Notch, suggesting either that *fa*^{swb} was suppressed when coupled to the male-viable deficiency or that none of the 30 recombinants carried *wrv-4* coupled to *fa*^{swb}; however, the recombinant chromosomes survived as males in progeny tests and provided phenotypic information suggesting that *fa*^{swb} was present in some. The dilute-w^a fa^g females were either *y* (*wrv-4*) *fa*^{swb} *fa*^{g} *rb*/*w*^{a} *fa*^{g} or *y* (*wrv-4*) + *fa*^{g} *rb*/*w*^{a} *fa*^{g}. Because of the presence of *y* and *wrv-4* in both types of females, males were produced that were yellow (*y*), white (*w*) and expressed the bristle defect verticals (*vt*). The expected expression of roughest (*rst*) was masked by the rough, glossy expression of *fa*^{g}. In some progeny tests, males with the recombinant chromosome clearly expressed the accessory wing-vein phenotype seen in the double mutant *fa*^{swb} *fa*^{g}; in other progeny tests, the wing-vein phenotype was totally absent. The accessory mutant phenotype indicated that the recombinant chromosome was *y* (*wrv-4*) *fa*^{swb} *fa*^{g} *rb*, and in the absence of it, the recombinant was *y* (*wrv-4*) + *fa*^{g} *rb*. Of the 30 recombinants, 23 expressed the wing-vein phenotype in male offspring; of these, nine were examined cytologically, and all had coupled deletions. Seven did not produce male progeny showing the accessory phenotype; we examined three, and only the *wrv-4* deletion was present (see Figures 1 and 2).

It was clear from the cytology and genetics that we had isolated the coupled deletions as *y* (*wrv-4*) *fa*^{swb} *fa*^{g} *rb* chromosomes; however, the expression of the accessory wing-vein phenotype seen in the double mutant *fa*^{swb} *fa*^{g} indicated that *fa*^{swb} was not suppressed, whereas the failure to observe an enhanced Notch phenotype in *y w*^{a} N^{55e11}/*y* (*wrv-4*) *fa*^{swb} *fa*^{g} *rb* females suggested that it was. The problem was not resolved until *fa*^{g} was removed from double-deletion chromosomes in the type B crosses.

For the male-viable deficiency *rst*^{2}, the mutant *w* was separable from *rst*^{2}, yielding an additional recombinant class that produced dilute-w^a fa^g females. Nevertheless, we were able to identify the three different recombinant chromosomes by examination of the males that carried them and confirm the identification cytologically. In 22,800 progeny, we isolated 21 recombinants. There were 11 *y w rst*^{2} *fa*^{swb} *fa*^{g} *rb* expressing the accessory phenotype. Seven recombinants resulted from exchanges between *w* and *rst*; three were crossovers between *fa*^{swb} and *fa*^{g}. We did not produce heterozygotes of N^{55e11} with the pertinent recombinant chromosomes.

When the coupled deficiency chromosomes were available, we performed crosses to eliminate the *fa*^{g} mutant. This would allow a phenotypic examination

TABLE 1

Recombination values obtained between fa^{swb} and five aberrations

Aberration	Experiment I	Experiment II	I + II
wrv-4	31/35,300 0.176	35/24,000 0.292	0.223
rst^2	25/24,000 0.208	22/27,000 0.163	0.184
In(1)78b[a]	30/51,900 0.116		0.116
wrv-7	20/51,300 0.078	19/42,800 0.089	0.083
wrv-6	6/41,900 0.029	9/47,300 0.038	0.034

Data are presented for two experiments separately (I and II) and in summation (I + II). Recombinants/tested chromosomes times two equals the map distance.

[a] Only one experiment performed.

of the coupled deletions free of interference caused by the expression of fa^g. In addition, we had discovered the genetic nature of *Dp(1;2)76f*, a derivative of *Dp(1;2)51b7* lacking the *N* to *dm* region but covering *w*, *rst* and *vt* to the left of *N*. For example, the male-lethal w^{67k30}, deficient for bands 3C2-6, survives as a normal male with *Dp(1;2)76f*, whereas w^{67k30} fa^g; *Dp(1;2)76f*/+ males express fa^g.

Type B, male-lethal crosses:

$$\frac{y\ (wrv\text{-}6)\ fa^{swb}\ fa^{g}\ rb}{+\quad +\qquad +\ +\ +} \times w^{a}\ fa^{g}\ rb$$

Recombination between *wrv-6* and fa^{swb} will produce a chromosome *y* (*wrv-6*), and when heterozygous with w^a fa^g *rb*, the females will be dilute-w^a. An exchange between fa^{swb} and fa^g produces *y* (*wrv-6*) fa^{swb}, and when heterozygous with w^a fa^g *rb*, the females will be dilute-w^a if fa^{swb} is suppressed; otherwise, they will be dilute-w^a and have rough, not-glossy eyes like fa^{swb}/fa^g heterozygotes. We also recorded the number of fa^{swb} fa^g *rb* males representing the reciprocal recombinant between *wrv-6* and fa^{swb} to estimate the frequency of exchange between the two deletions. The values are shown in Table 1 for this and the ensuing crosses.

The cross produced 31,400 progeny. Because one-quarter of the zygotes do not survive due to the lethality of *wrv-6*, we estimate 41,900 tested chromosomes for calculating the crossover value. Eleven dilute-w^a females were produced, and none had rough eyes. They were progeny tested by mating to w^{67k30} fa^g;*Dp(1;2)76f*/+ males, and none of the male progeny with the recom-

binant chromosome and the *Dp* expressed fa^{swb}. All were examined cytologically; seven had *wrv-6*-fa^{swb} cytology representing the *y* (*wrv-6*) fa^{swb} recombinant chromosome, and four had *wrv-6* cytology representing the *y* (*wrv-6*) recombinant (Figures 1 and 2). It was clear that, when coupled to the male-lethal deficiency, fa^{swb} was suppressed. The analysis of the male-lethal *wrv-7* proceeded in the same way and resulted in the same conclusion. In 38,500 progeny (51,300 tested chromosomes), we found 11 *y* (*wrv-7*) recombinants and five *y* (*wrv-7*) fa^{swb} chromosomes with fa^{swb} suppressed.

Type B, male-viable crosses:

$$\frac{y\ (wrv\text{-}4)\ fa^{swb}\ fa^{g}\ rb}{+\quad w^{a}\quad +\ +\ +} \times w^{a}\ fa^{g}\ rb$$

Although w^a was present in this cross, the method of analysis was unchanged. We isolated 14 dilute-w^a recombinant females, and none expressed a rough-eye phenotype. When tested by mating to w^{67k30} fa^{g};*Dp(1;2)76f*/+, all males with the recombinant chromosome and *Dp* expressed only *y*. This could mean that all were derived from an exchange between *wrv-4* and fa^{swb} yielding only *y* (*wrv-4*) chromosomes; however, cytological analysis revealed that one was a *y* (*wrv-4*) fa^{swb} recombinant and that 13 had *y* (*wrv-4*) chromosomes (Figure 1 and 2). In *cis* with *wrv-4*, the eye phenotype of fa^{swb} was suppressed, but the wing phenotype resulting from the interaction between fa^{swb} and fa^{g} was unchanged.

The analysis of the male-viable rst^2 deletion yielded 14 *y w* rst^2 and ten *y w* rst^2 fa^{swb} recombinants. None of the 24 expressed a rough-eye phenotype with w^a fa^g *rb*, and in males with the *Dp*, only the mutant *y* was expressed. The eye phenotype of fa^{swb} was suppressed in *cis* with rst^2, but the accessory phenotype was not, and anticipating some data yet to be presented, we can state that the situation is the same for each of the four deficiencies.

The type C crosses were performed to verify the expectation that we could recover fa^{swb} by uncoupling the double deletions. The double deficiency *y* (*wrv-6*) fa^{swb} is used as a demonstration.

Type C crosses:

$$\frac{y\ (wrv\text{-}6)\ fa^{swb}\ +}{+\quad +\quad +\ rb} \times w^{a}\ fa^{g}\ rb$$

Recombination between *wrv-6* and fa^{swb} will yield fa^{swb} males and fa^{swb}/w^a fa^g *rb* heterozygotes with rough eyes. Recombination values are shown in Table 1 for all four crosses. The data demonstrate that the coupled deletions necessarily recognized by cytological examination in the type A crosses were correctly identified.

Deficiencies in the *w-N* region are not the only aberration that suppresses the eye phenotype of fa^{swb}. D. O. KEPPY irradiated fa^{swb} males and isolated a reversion that carried *In(1)3A2-3;3C3-5* and the fusion of 3C6 with 7 representing fa^{swb} cytology. The two aberrations can be separated by crossing over as demonstrated in the cross:

$$\frac{w^{a}\quad +\ fa^{g}\ rb}{(In\ +)\ fa^{swb}\ +\ +} \times w^{a}\ fa^{g}\ rb$$

The symbol (*In* +) indicates the w^+ condition of the inversion to the left of fa^{swb}. Because the inverted *w* locus is shifted distally to the neighborhood of zeste (*z*), recombination between the inversion and w^a does not occur. Crossing over can occur in the region between the inversion and fa^{swb} and between fa^{swb} and fa^g. We attempted to isolate only the recombinant chromosomes (*In* +) + fa^g *rb* and (*In* +) fa^{swb} fa^g *rb*. Both are phenotypically fa^g in heterozygotes with the parental chromosome, and hemizygous males express fa^g in one case and, in the other, show the accessory phenotype in addition to fa^g. We isolated 30 (*In* +) + fa^g *rb* and 14 (*In* +) fa^{swb} fa^g *rb* chromosomes expressing the double mutant phenotype characteristic of fa^{swb} fa^g and confirming the presence of fa^{swb} in a suppressed condition on the inversion chromosome. The recombination values are shown in Table 1.

Once we were aware that the eye phenotype of fa^{swb} (but not the accessory phenotype of the double mutant) was suppressed in *cis* with the deficiencies, we rechecked them all. As an example, we can use *wrv-6* to summarize the situation. (1) *y* (*wrv-6*) fa^{swb}/fa^g heterozygotes do not express the rough-eye phenotype seen in fa^{swb}/fa^g females; (2) *y* (*wrv-6*) fa^{swb};*Dp(1;2)76f*/+ males do not express fa^{swb}; (3) *y* (*wrv-6*) fa^{swb} fa^g;*Dp(1;2)76f*/+ are phenotypically fa^g and express the accessory phenotype; (4) *y* (*wrv-6*) fa^g;*Dp(1;2)76f*/+ expresses only fa^g. To the four deficiencies used here, we can add the case of w^{67k30}, the deletion in which we first noticed the suppression of fa^{swb}. The genotypes (1) to (4) produce identical results. With *In(1)78b*, we do not have to use *Dp(1;2)76f* to make the same observations. Hemizygotes fa^{swb};*Dp(1;2)76f* express fa^{swb}.

DISCUSSION

The recombination values between the fa^{swb} deletion and the five aberrations distal to it are found in Table 1. In these crosses, the male-viable deficiencies *wrv-4* and rst^2 probably are underrepresented as a surviving class of progeny, whereas in crosses with *wrv-6* and *wrv-7*, one class is completely absent. Comparisons between the male-lethal crosses probably are more reliable than between the male-viable crosses, and comparisons between the two categories are likely to be the least reliable.

The map distances between each of the deficiencies and fa^{swb} are produced by crossing over between the proximal breakpoint in a *wrv* mutant (or rst^2) and the distal break in the fa^{swb} deletion (see Figure 2). For the male-viable deficiencies, in the summation of two crosses, the greatest map distance was found between *wrv-4* and fa^{swb}, although in one case, the rst^2 to fa^{swb} value was greater. We doubt that a comparison of these values can seriously support one cytological definition of *wrv-4* over another. For the male-lethal crosses, *wrv-6* to fa^{swb} had the shortest map distance. If we express the smallest value found in these experiments as 290×10^{-4} map units and compare it with the largest value of 13×10^{-4} obtained for w^{67k30} to fa^{swb} (KEPPY and WELSHONS 1977), we find the distance *wrv-6* to fa^{swb} is some 22 times greater than w^{67k30} to fa^{swb}. The comparatively large map distance seen with *wrv-6* might signify that, whereas both *wrv-6* and w^{67k30} were defined as deficiencies for 3C2-6 (LEFEVRE and GREEN 1972), *wrv-6* might yet retain some part of 3C6 contributing to

the map distance *wrv-6* to fa^{swb}. If 3C6 is present in part in *wrv-6*, then it probably is present in *wrv-7* because the map distance to fa^{swb} is even greater.

The genetic distance between the inversion breakpoint and the deletion in fa^{swb} was 0.12, a value intermediate between the male-viable and male-lethal deficiencies. We could have expected to see a value approximating 0.7, equal to about half the *w* to *N* interval. Relatively large inversions suppress the frequency of exchange in normal sequences adjacent to the breakpoint (GRELL 1962; ROBERTS 1962), but a tiny inversion between 3C7 and 3C10 (N^{76b8}) that was used to enter the locus for the molecular cloning of Notch (ARTAVANIS-TSAKONAS, MUSKAVITCH and YEDVOBNICK 1983) had no noticeable effect on crossing over in intact adjacent sequences (WELSHONS and KEPPY 1981). *In(1)78b* is neither tiny nor large, and perhaps some inhibition to exchange is occurring adjacent to the breakpoint.

KIDD, LOCKETT and YOUNG (1983) found that N^{55e11} is associated with a 3.5-kb insertion at the 5′ end of the Notch locus. Recently, GRIMWADE *et al.* (1985) have shown that fa^{swb} is a 0.8 kb deletion of sequences between the proximal limit of the deficiency in w^{67k30} and the distal limit of the insertion at N^{55e11}. In addition, it is unlikely that the deleted sequences in fa^{swb} are complementary to a 10.5-kb poly(A)$^+$ RNA product of Notch, although the data on this point are not perfectly clear. The deletion in fa^{swb}, its position at the 5′ end of the locus and its location between w^{67k30} and N^{55e11} are fully concordant with genetic and cytological data. One might suggest that the resumption of Notch function, observed when fa^{swb} is placed in *cis* with a distal aberration, results from a juxtaposition of distant sequences to the 5′ end of Notch, and by means of promotor fusion, function has been restored. It is unlikely, however, that each of six different aberrations would result in such fortuitous fusions (GRIMWADE *et al.* 1985). For the present, we will presume that fa^{swb} is a position effect caused by a small deletion that allows distal functions to interfere with activity at the Notch locus, thus producing the fa^{swb} mutant; when the interfering functions are eliminated, the aberrant condition is suppressed.

Of the five deficiencies we have used, four of them either totally eliminate 3C5,6 (w^{67k30}) or have breakpoints in it (rst^2, *wrv-6*, *wrv-7*). Either *wrv-4* has a breakpoint in 3C5,6 as originally claimed or, if it is a simple deletion in 3C2,3, it has, in addition, a disruption of function in 3C5,6 giving it the *rst* and *vt* phenotype (LEFEVRE and GREEN 1972). Add to the deficiencies *In(1)78b* with a breakpoint in the interval 3C3-5 and it seems that, in the presence of fa^{swb}, chromosomal breaks proximal to 3C3 and distal to 3C7 can eliminate interfering processes adjacent to 3C7, thereby allowing resumption of normal functions at Notch; perhaps aberration breakpoints even more distally located will also suppress fa^{swb}, but we have no data that say so.

SHELLENBARGER and MOHLER (1975, 1978) have identified the temperature-sensitive periods for lethality and aberrant morphological phenotypes at Notch; more recently, JIMENEZ and CAMPOS-ORTEGA (1982) have demonstrated a maternal effect. Developmental profiles for the poly(A)$^+$ RNA at Notch parallel the genetic data, within experimental error, suggesting transcriptional control

of Notch expression (ARTAVANIS-TSAKONAS, MUSKAVITCH and YEDVOBNICK 1983; KIDD, LOCKETT and YOUNG 1983; ARTAVANIS-TSAKONAS *et al.* 1984); hence, a consideration of the position effect with recourse to the temperature-sensitive studies might be instructive. If we presume that the deletion in fa^{swb} represents a loss of DNA sequences that ordinarily insulate the locus from adjacent functions, then the condition at Notch becomes an assay for genetic activity in the *w* to *N* region. As a demonstration, we will make a deliberate simplification. Presume that the Notch locus can either be fully functional or completely inactive. With this restriction, every mutant condition results from the lack of function, and one must explain why it is that the amorphic condition at Notch is a recessive lethal when we consider *N* mutants and a viable recessive visible in the case of fa^{swb}. The apparent contradiction can be explained by assuming that, when the lack of activity at Notch causes the visible mutant effect, the vital requirements of Notch function have already been met on time and in the appropriate tissues of an earlier developmental stage. Lack of activity at a later time and in the tissues that contribute to the formation of a normal eye does not result in lethality. It leads, instead, to the development of a less than perfect eye recognized as the fa^{swb} phenotype. Because there is no lethal condition associated with fa^{swb}, the Notch locus must be functional in the embryo, second and third instars and in prepupal stages for which lethal temperature-sensitive periods have been found (SHELLENBARGER and MOHLER 1975, 1978). The implication follows that the interfering functions must be quiescent at these specific times. The noncomplementarity of fa^{swb}/fa^{g} heterozygotes suggests that the mutants have overlapping TSPs in the early pupal stage (SHELLENBARGER and MOHLER 1975, 1978); hence, the adjacent region distal to Notch becomes active at this time, causing the developmental abnormality recognized as the fa^{swb} phenotype.

Problems with the interpretation arise when we consider the results obtained with *In(1)78b*. In this aberration, bands 3C1 to 3 are separated from 3C5,6 and 7 which remain in normal sequence. Because the eye phenotype of fa^{swb} is not expressed in *cis* with the inversion, we would expect 3C5,6 to be inactive in the early pupae so as not to interfere with a Notch locus function. One might expect lack of function in 3C5,6 to produce a mutant condition *rst* and/or *vt* as do the deletions, but we have not seen a mutant phenotype associated with the inversion. The study by LEFEVRE and GREEN (1972) demonstrated that a deletion for 3C6 heterozygous with a deficiency for bands 3C2-6 (N^{54l9}/w^{67k30}) was mutant for *vt*. If 3C5,6 is inactive in *In(1)78b*, we could expect $In(1)78b/w^{67k30}$ heterozygotes to express a mutant phenotype, but they do not; the *In(1)78b* does not express a mutant phenotype as a hemizygote, homozygote or in heterozygotes with w^{67k30}.

It might be that the functions of 3C5,6 are not eliminated in *In(1)78b*, but they may have been altered in some way that cannot be discerned by visual examination. One might then suggest that the deletion in fa^{swb} alters the response of the Notch locus such that with one set of conditions the elimination of adjacent genetic functions is required for the suppression of the mutant phenotype, whereas at different times and places in development, alterations

in adjacent genetic activity allow Notch locus function. Alternatively, it might be that the activity of the region between 3C3 and 6 is capable of causing a hypomorphic state at Notch rather than, or in addition to, the amorphic condition that we imposed as a restriction when we first considered the position effect in relation to the temperature sensitivity studies. If partial activity at Notch is a consequence of the interference caused by adjacent functions, observing the condition at Notch might not constitute an accurate assay for them.

To depart entirely from the idea that linked functions influence Notch functions, one might say that the deletion in fa^{swb} causes a position effect upon the Notch locus, and more distal aberrations exert a position effect upon fa^{swb}, thus reversing the mutant effect. With this interpretation, the chromosomal disruptions could be the cause of the mutant condition and its suppression rather than genetic activity adjacent to Notch. We will continue to interpret the position effect at the functional level until this view becomes untenable.

That the deleted sequences in fa^{swb} do more than provide insulation and may alter Notch function independent of adjacent functions is suggested by the fact that six different aberrations in *cis* with fa^{swb} suppress the eye phenotype, whereas none suppress the wing effect in the fa^{swb} fa^{g} double mutant. Perhaps we are seeing an effect of the deletion on the action of the 5-kb *copia*-like insertion that is the probable cause of the fa^{g} mutant expression (KIDD, LOCKETT and YOUNG 1983). The deleted sequences in fa^{swb} may have a subtle effect upon Notch (even when fa^{swb} is suppressed) that is ordinarily undetectable. When the foreign element is present in the host locus, the slight change in Notch function alters the action of the foreign element, resulting in the development of the accessory phenotype to accompany the mutant-eye effect—two aberrant morphological conditions with overlapping TSPs (SHELLENBARGER and MOHLER 1978). It would mean that the 0.8-kb deletion has two observable effects: one is a modifiable position effect resulting in the fa^{swb} rough-eye phenotype; the other is a stable effect on the action of foreign DNA inserted in the host locus.

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LITERATURE CITED

ARTAVANIS-TSAKONAS, S., B. G. GRIMWADE, R. G. HARRISON, K. MARKOPOULOU, M. A. T. MUSKAVITCH, R. SCHLESINGER-BRYANT, K. WHARTON and B. YEDVOBNICK, 1984 The Notch locus of *Drosophila melanogaster*: a molecular analysis. Dev. Genet. **4:** 223–254.

ARTAVANIS-TSAKONAS, S., M. A. T. MUSKAVITCH and B. YEDVOBNICK, 1983 Molecular cloning of Notch, a locus affecting neurogenesis in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **80:** 1977–1981.

COLLINS, M. and G. M. RUBIN, 1982 Structure of the *Drosophila* mutable white-crimson and its white-ivory and wild-type derivatives. Cell **30:** 71–79.

GREEN, M. M., 1967 The genetics of a mutable gene at the white locus of *Drosophila melanogaster*. Genetics **56:** 467–482.

GRELL, R. F., 1962 A new model for secondary nondisjunction: the role of distributive pairing. Genetics **47:** 1737–1754.

GRIMWADE, B. G., M. A. T. MUSKAVITCH, W. J. WELSHONS, B. YEDVOBNICK and S. ARTAVANIS-TSAKONAS, 1985 Molecular genetics of the Notch locus in *Drosophila melanogaster*. Dev. Biol. **107:** 503–519.

JIMENEZ, F. and J. A. CAMPOS-ORTEGA, 1982 Maternal effects of zygotic mutants affecting early neurogenesis in Drosophila. Wilhelm Roux Arch. **191:** 191–201.

KEPPY, D. O. and W. J. WELSHONS, 1977 The cytogenetics of a recessive visible mutant associated with a deficiency adjacent to the Notch locus in *Drosophila melanogaster*. Genetics **85:** 497–506.

KEPPY, D. O. and W. J. WELSHONS, 1980 The synthesis of compound bands in *Drosophila melanogaster* salivary gland chromosomes. Chromosoma (Berl.) **76:** 191–200.

KIDD, S., T. J. LOCKETT and M. W. YOUNG, 1983 The Notch locus of *Drosophila melanogaster*. Cell **34:** 421–433.

LEFEVRE, G., JR., 1952 *Dp(1;2)w*^{51b7}. Drosophila Inform. Serv. **26:** 66.

LEFEVRE, G., JR. and M. M. GREEN, 1972 Genetic duplication in the white-split interval of the X chromosome in *Drosophila melanogaster*. Chromosoma (Berl.) **36:** 391–412.

LEFEVRE, G., JR. and J. KELLEY, 1972 Strawberry vs. facet-glossy, a locus correction. Drosophila Inform. Serv. **48:** 146–147.

LEVIS, R., M. COLLINS and G. M. RUBIN, 1982 FB elements are the common basis for the instability of the *w*^{DZL} and *w*^{e} *Drosophila* mutations. Cell **30:** 551–565.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

ROBERTS, P., 1962 Interchromosomal effects and the relation between crossing over and nondisjunction. Genetics **47:** 1691–1709.

SHELLENBARGER, D. L. and J. D. MOHLER, 1975 Temperature-sensitive mutations of the Notch locus in *Drosophila melanogaster*. Genetics **81:** 143–162.

SHELLENBARGER, D. L. and J. D. MOHLER, 1978 Temperature-sensitive periods and autonomy of pleiotropic effects of *l(1)N*$^{ts-1}$, a conditional Notch lethal in *Drosophila*. Dev. Biol. **62:** 432–446.

SORSA, V. and A. O. SAURA, 1980 Electron microscopic analysis of the banding pattern in the salivary gland chromosomes of *Drosophila melanogaster*: divisions 3, 4 and 5 of X. Hereditas **92:** 341–351.

WELSHONS, W. J. and D. O. KEPPY, 1975 Intragenic deletions and salivary band relationships in Drosophila. Genetics **80:** 143–155.

WELSHONS, W. J. and D. O. KEPPY, 1981 The recombinational analysis of aberrations and the position of the Notch locus on the polytene chromosome of *Drosophila*. Mol. Gen. Genet. **181:** 319–324.

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