THE CYTOGENETICS OF A RECESSIVE VISIBLE MUTANT ASSOCIATED WITH A DEFICIENCY ADJACENT TO THE NOTCH LOCUS IN *DROSOPHILA MELANOGASTER*[1]

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Manuscript received November 15, 1976

ABSTRACT

The recessive visible fa^{swb} allele in Drosophila is an interband deletion between salivary band 3C5, 6 and 7. Heterozygosity for the deletion does not suppress recombination between fa^{swb} and mutant sites at Notch adjacent to it.—*Df(1)*w^{67k30}, deficient for salivary bands 3C2 to 6, is to the left of fa^{swb}. By crossing over within the homologous bit of interband retained in w^{67k30} and fa^{swb}, the two deficiencies can be linked. Cytologically, 3C7, "fused" to 3C5, 6 in fa^{swb}, becomes "fused" to 3C1 when the two are coupled. In the double deletion, the recessive visible phenotype of the fa^{swb} "allele" is suppressed. Both w^{67k30} and fa^{swb} can be recovered by uncoupling the two deficiencies.—The data suggest that the mutant fa^{swb} does not represent a lesion at Notch; the entire gene or locus seems to be present. The interband deletion in fa^{swb} has secondarily moved an intact Notch locus to a foreign environment that interferes with its normal function. When fa^{swb} is linked to w^{67k30}, the interference is eliminated and normal Notch functions resume.—The position of Notch on the salivary gland chromosome is reviewed in relation to the information obtained in these experiments.

EARLIER, we described the genetics and cytology of a recessive visible mutant called facet-strawberry (fa^{swb}). It was localized to the *X*-linked Notch locus of Drosophila and associated with a small chromosomal aberration (WELSHONS and KEPPY 1975). From our examinations, we considered three possible interpretations of its cytology and, subsequently, eliminated two on the basis of cytogenetic information. The third and favored view interpreted the aberration as a small deficiency generated by one breakpoint immediately to the right of salivary band 3C5,6 and the other at the left edge of 3C7. The resulting deletion was largely interband 3C5,6 to 7, although there could have been some loss of 3C7 at the very left edge of that band.

Our continued cytogenetic investigation of fa^{swb} has provided data strongly supporting the cytological definition of the deficiency and, simultaneously,

[1] Journal Paper No. J-8455 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 1985. Work supported in part by National Science Foundation grants GB-8651 and GB-30744.

[2] This work is in part from the thesis submitted by D. O. Keppy in partial fulfillment of the degree of Doctor of Philosophy at Iowa State University. Present address: Division of Biology, Kansas State University, Manhattan, Kansas 66506.

giving evidence that fa^{swb}, although behaving like an allele at Notch, is not a lesion in the Notch gene. There is no need to presume that any part of the gene or locus is missing. It seems that a deletion immediately to the left of the intact locus has secondarily placed Notch in association with a foreign environment that interferes with normal Notch locus function and results in the recessive visible mutant effect recognized as fa^{swb}.

MATERIALS AND METHODS

The recessive visible allele facet-strawberry (fa^{swb}) was X-ray induced by LEFEVRE and KELLEY (1972), who demonstrated its allelism at the Notch locus. The mutant is characterized in males by an abnormal eye facet arrangement resulting in a rough-eye phenotype with some variable tendency to be slightly glossy at scattered spots. Concerning more subtle effects, fa^{swb} shows extra and irregularly aligned microchaetae on the thorax causing a slightly "hairy" appearance characteristic of some Notch alleles. Wing vein III is slightly thickened and there are occasional small deltas at the juncture of longitudinal veins with the marginal vein. The fa^{swb} allele is not dosage compensated; hence, the mutant phenotype is diminished in homozygous females and overlaps wild type. The main phenotypic criterion used to judge the fa^{swb} allele during recombination experiments has been the rough-eye effect.

The recessive visible fa^{swb} is associated with a cytological aberration which at first was thought to be a deficiency for salivary band 3C7. Continued cytogenetic efforts led us to believe that the deficiency was primarily a deletion of the interband between 3C5, 6 and 7; the band 3C7 was fused to 6, causing 3C5, 6 to look thick and protuberant while 7 seemed to be missing (WELSHONS and KEPPY 1975).

Besides the recessive visible fa^{swb}, there are six other recessive alleles at Notch that have been used in recombination experiments. They can be separated into the classes of eye (facet, *fa*; facet-glossy, fa^{g}; split, *spl*) and wing (facet-notchoid, fa^{no}; notchoid, *nd*; notchoid-2, nd^{2}) mutants, all of which show a pseudodominant expression when heterozygous with a *N* allele. Females fa^{swb}/fa^{g} are noncomplementary in that the eyes are rough and exhibit a mottled-glossy effect characteristic of fa^{swb} males. Heterozygotes fa^{swb}/fa^{no} occasionally show wing vein deltas; all other heterozygotes of fa^{swb} with recessive visible alleles at Notch are visibly complementary.

The mutant fa^{swb} shows an extreme pseudodominant expression when heterozygous with amorphic recessive lethal *N* alleles, and these N/fa^{swb} females are semilethal. To enhance the viability of N/fa^{swb} heterozygotes in recombination experiments, the duplication *Dp(1;2)51b7* (LEFEVRE 1952) was used. The temperature-sensitive N^{60g11} is an exception in that N^{60g11}/fa^{swb} females do not require the duplication to enhance viability and fertility.

The recessive lethal Notch (*N*) alleles, viewed as point mutants, are linearly arranged in the order N^{55e11}, N^{264-40}, N^{Co}, and N^{60g11}. The deficiency Notch, $Df(1)N^{62b1}$, was induced by

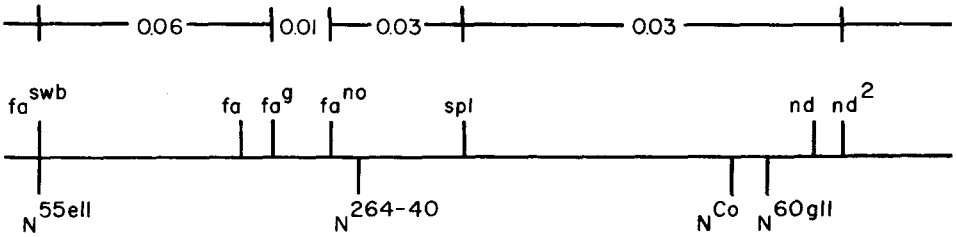

FIGURE 1.—Genetic map of the Notch locus. The recessive visible alleles are shown above the line and some selected recessive lethal Notch mutants below the line.

irradiation and has been cytologically described as a deletion for bands 3C8–3D5. It lacks about 80% of the right portion of the genic map (WELSHONS 1974) and extends to the locus of diminutive (*dm*). Additional information relating to all *N* mutants (except the deficiency N^{62b1}) and all recessive visibles (except fa^{swb}) will be found in LINDSLEY and GRELL (1968).

The X-ray-induced deficiency $Df(1)w^{67k30}$ is a deletion for salivary bands 3C2–3C6. Genetically, it is male lethal and lacks the normal white (*w*), roughest (*rst*) and verticals (*vt*) functions (LEFEVRE and GREEN 1972).

The methods of genetic analysis have been previously reported (WELSHONS 1974; WELSHONS and KEPPY 1975), and, as before, temporary salivary gland chromosome preparations were made with a lactic-acetic-orcein squash technique.

RESULTS

Recombination between fa^{swb} *and mutant sites at Notch*: We have obtained recombination values between fa^{swb} and all of the recessive visible mutants represented on the genetic map (Figure 1). In addition, each of the six double mutant combinations has been isolated. The experiments place fa^{swb} to the left of *fa* at the left-most end of the genetic map, along with N^{55e11} (Figure 1).

The failure to obtain recombinants between fa^{swb} and N^{55e11} has already been reported (WELSHONS and KEPPY 1975). More recently we have measured recombination between fa^{swb} and the recessive lethal sites N^{Co} and N^{60g11} at the right end of the map. The crossover values were 0.29 and 0.28, respectively, whereas we would have expected values of approximately 0.12 in both cases since N^{Co} and N^{60g11} are closely linked. Because recombinants were not obtained from heterozygotes of fa^{swb} and N^{55e11}, it seemed that the recessive visible was at or close to this *N* site; hence, fa^{swb} should recombine with the deficiency N^{62b1}, as did N^{55e11}, because $Df(1)N^{62b1}$ retains a bit of the Notch gene in the region N^{55e11} to *fa* (Figure 1). It has been shown that recombination does occur (WELSHONS and KEPPY 1975).

The totality of our recombination experiments places fa^{swb} at the far left of the map, and from heterozygotes of fa^{swb} with eight different mutant sites at Notch, we have consistently obtained map distances equal to or greater than expected. Remembering that fa^{swb} is believed to be a small deficiency immediately adjacent to the Notch sites in which we are measuring crossing over, and noting that in each of the eight crosses the deficiency was heterozygous, we were surprised to find normal to high recombination values when we expected to see evidence of suppression. Variation in estimates can be expected because of the necessity to use unstandardized methods to measure map distances, but these data provide not a single hint that heterozygosity for the deficiency has diminished crossing over as a consequence of pairing problems expected to exist at the time of exchange.

Recombination between w^{67k30} *and* fa^{swb}: Viewing the fa^{swb} mutant as a cytological deletion between salivary bands 3C5,6 and 7, we wished to see if it would recombine with $Df(1)w^{67k30}$, defined as a deficiency for bands 3C2 to 3C6 (LEFEVRE and GREEN 1972). Representing w^{67k30} as w^-, we performed the cross

$$\frac{w^- \; + \; fa^g}{+ \; fa^{swb} \; +} \times \frac{w^a + fa^g}{Y}$$

and hoped to find crossovers between w^- and fa^{swb} resulting in $w^-\ fa^{swb}\ +$ and $+\ +\ fa^g$ chromosomes. The double mutant, which would survive only in the female progeny, would actually represent a coupling of two closely linked deletions. That crossing over could occur between w^- and fa^{swb} was demonstrated by the isolation of two $+\ +\ fa^g$ reciprocal recombinants, but females carrying the $w^-\ fa^{swb}\ +$ chromosome were not detected (WELSHONS and KEPPY 1975). The two $+\ +\ fa^g$ recombinants had normal cytology indicating that the aberrancies in w^- and fa^{swb}, viewed collectively, had the material from which a visibly normal chromosome could be reconstructed by crossing over.

For cytological purposes, we had retained a sample of recombinants between the alleles fa^{swb} and fa^g. In two cases, male larvae of the genotype $+\ fa^{swb}\ fa^g$ had fa^{swb} cytology as expected. Of three $w^-\ +\ +$ recombinants similarly examined, two had the typical cytology of w^- in which 3C1, 3C7, and 3C9,10 are clearly visible, but a third seemed to have only two bands instead of three. We referred to the recombinant with the unexpected cytology as w^{-26D} and subjected it to genetic tests. Like w^- $(=w^{67k30})$, w^{-26D} is lethal in males and lacks w, rst and vt functions. Notch functions are intact in that both $w^-/+$ and $w^{-26D}/+$ are phenotypically normal as are w^-/fa^g and w^{-26D}/fa^g, and either deficiency heterozygous with N^{55e11} is indistinguishable from $N^{55e11}/+$.

The $Df(1)w^{67k30}$ retains the prominent bands 3C1, 3C7 and 3C9,10. In our preparations, 3C1 and 3C7 are approximately equal in size and staining properties, whereas 3C9,10 is a bit heavier (Figure 2). The w^{-26D} deletion seemed to have only two bands, an exceptionally heavy one in the 3C1 position, and a band of lesser density in the position we took to be 3C9,10 (Figure 2D). Larvae of the genotype w^-/w^{-26D}, incorporating $Dp(1;2)51b7$ to cover the lethality, were prepared and examined cytologically. We concluded that the fainter of the two bands in w^{-26D} was 3C9,10 in that it paired regularly with 3C9,10 in w^-. The heavy single band in w^{-26D}, however, seemed to pair irregularly with 3C1 or 3C7 in w^-. The examination convinced us that w^{-26D} had an intact 3C9,10; it also obliged us to consider that the heavy band represented a fusion of 3C1 with 3C7 resulting in a single entity larger than 3C9,10.

Suspecting that the origin of w^{-26D} might be an event deserving of more attention, we decided to look for it anew in an experiment that had already been initiated for another purpose. The cross was made as indicated:

$$\frac{w^-\ +\ N^{60g11}\ +}{+\ fa^{swb}\ +\ rb} \times \frac{w^a\ fa^g\ fa^{no}\ rb}{Y}$$

The $w^-\ +\ +\ rb$ chromosomes are recombinants between fa^{swb} and N^{60g11}, and it was in this sample that we hoped to find another w^{-26D}, but $w^{-26D}\ +\ +\ rb$ and $w^-\ +\ +\ rb$ could be differentiated only by cytological examination. There were 91 recombinant chromosomes in the sample and one was w^{-26D}. The data suggest two things: (1) The origin of w^{-26D} is a rare repeatable event associated with crossing over in heterozygotes of w^- with fa^{swb}. (2) The cytological difference between w^- and w^{-26D} is small but readily discerned.

At this point, we seriosuly considered that w^{-26D} did represent the deficiencies w^- and fa^{swb} coupled together in *cis*, but that fa^{swb} was not expressed. The assump-

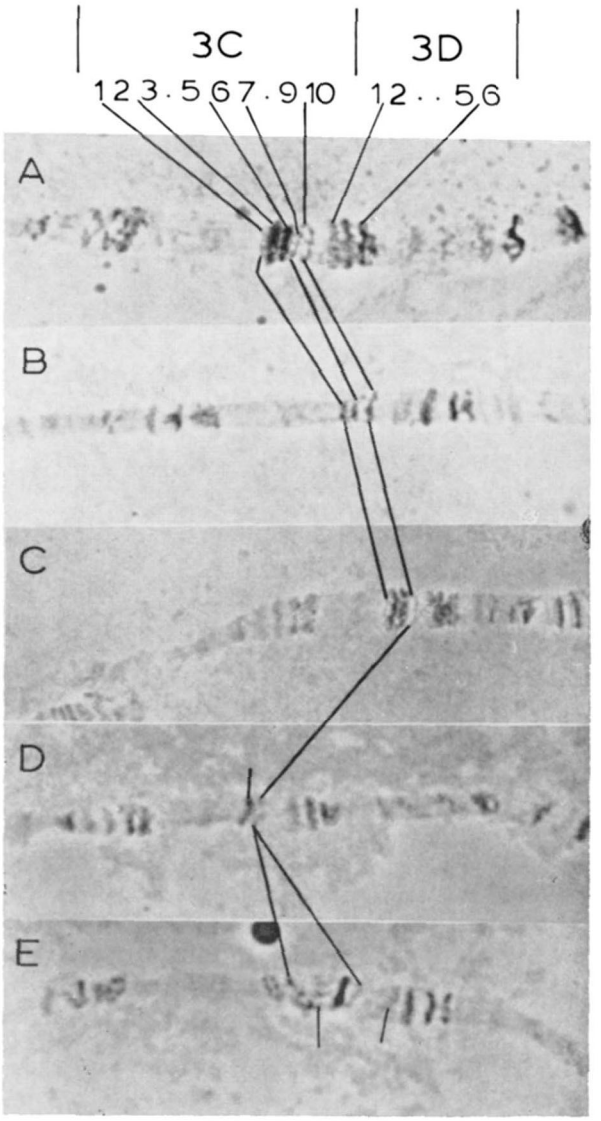

FIGURE 2.—Salivary gland preparations of *X* chromosomes. A. The *X* chromosome of a wild-type (Canton-S) male. B. The *X* chromosome of a $Df(1)w^{67k30}$ male illustrating the absence of bands 3C2 through 3C6. C. The *X* chromosome of a fa^{swb} male illustrating the apparent absence of 3C7. D. The *X* chromosome of a w^{-26D} male. The pointer indicates the single band where normally 3C1 and 3C7 are found in $Df(1)w^{67k30}$. E. The *X* chromosome of a *w(id)* male showing two 3C9,10 bands and two 3D1,2 bands at the pointers. Band sequence starting with w^{-}: — 3C1, 3C7, to 3D1,2, 3C5,6 (fa^{swb}) to 3D1,2.

tion that fa^{swb} was suppressed seemed implausible, but we persisted because the origin of w^{-26D} cytology was readily explained by crossing over between w^{-} and fa^{swb} without any change in our view that the aberration in fa^{swb} was caused by an interband deletion between 3C5,6 and 7. Figure 3A demonstrates the het-

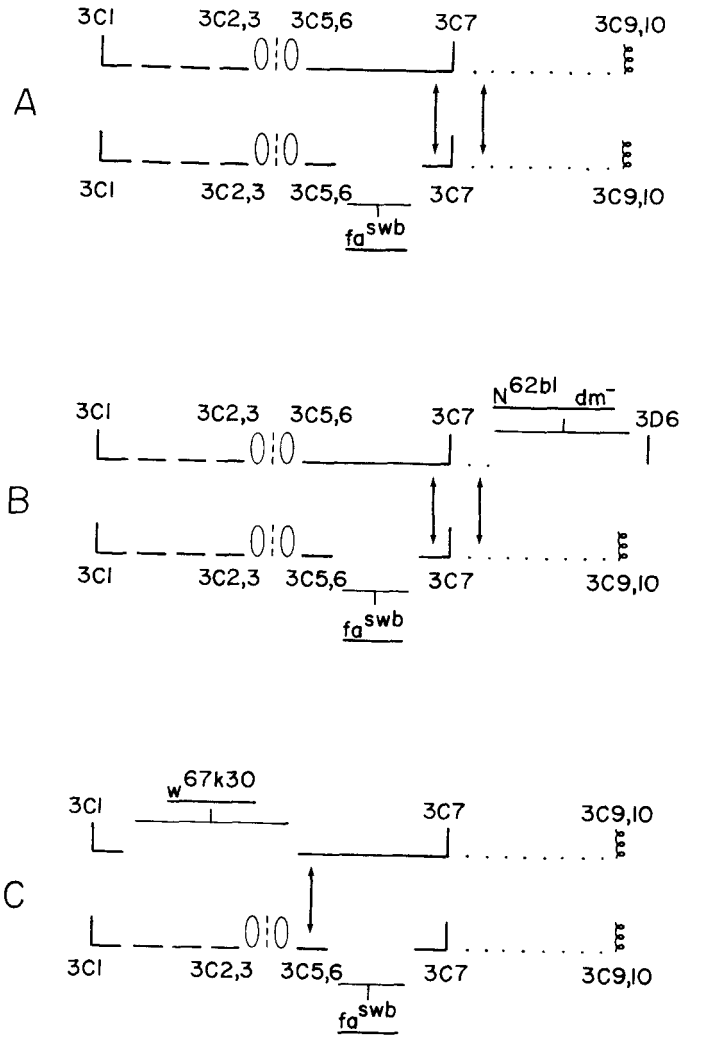

FIGURE 3.—Schematic representation of the chromosomes involved in the genetic experiments based on the assumption that fa^{swb} is an interband deletion between 3C6 and 7.

A. Cross between fa^{swb} and any other recessive visible or nondeletion *N* allele.

B. Cross between fa^{swb} and $Df(1)N^{62b1}$.

C. Cross between fa^{swb} and $Df(1)w^{67k30}$.

See text for discussion.

erozygous condition of fa^{swb} with a cytologically normal chromosome carrying any point mutant in the Notch gene believed to be to the right of 3C7 (WELSHONS and KEPPY 1975). Presuming that fa^{g} is on the normal chromosome, then crossing over within the limits of the vertical arrows will generate, as found, both a nonmutant condition with normal cytology and a double mutant fa^{swb} fa^{g} with fa^{swb} cytology. Figure 3B represents the heterozygous *trans* condition of fa^{swb} and $Df(1)N^{62b1}$ which yielded only nonmutant normal chromosomes, since the

experiment was designed to select against recessive lethal Notches (WELSHONS and KEPPY 1975). From Figure 3C, one can derive the consequences of exchange between the two deletions in w^-/fa^{swb} heterozygotes. In w^-, most of the interband 3C5,6 to 7 is retained; in fa^{swb} a bit of the same homologous interband adjacent to 3C5,6 is present, and some might be retained adjacent to 7. The relatively long stretch of interband in w^- presumably allows the cytological separation of 3C1 and 3C7, whereas the deletion of most of this same interband in fa^{swb} has placed 3C5,6 so close to 7 that the bands cannot be individually resolved (Figure 2). A crossover event at the arrow will generate a chromosome with normal cytology (as observed), and a reciprocal event in which 3C7, initially "fused" to 3C5,6 in fa^{swb}, becomes "fused" to 3C1 in w^-.

The diagrammatic representation allows the peculiar cytology of w^{-26D} to be generated by crossing over, but an additional assumption is required to explain the phenotypic suppression of fa^{swb} coupled to w^-. One might suspect that the genetics and cytology could be explained by viewing the nonexpression of fa^{swb} as due to the formation of a duplication following nonhomologous pairing and exchange. We have isolated duplications, called $w(id)$, that seem to have originated in this way (Figure 3E), but it is difficult to look upon w^{-26D} as the duplication product of an aberrant exchange mechanism, since it is an even larger deletion than w^-. We concluded that w^{-26D} represented a *bona fide* crossover product, and the suppression of the mutant phenotype had to be explained by a mechanism other than the genesis of a duplication.

The recovery of w⁻ *and* fa^{swb} *from* w^{-26D}: If w^{-26D} represents the coupled deficiencies, we should be able to uncouple them with predictable results. Crossing over in appropriate heterozygotes should yield recombinant progeny expressing the fa^{swb} phenotype, and the isolated mutant chromosome should have fa^{swb} cytology (Figure 2). The reciprocal recombinant should yield a chromosome that is genetically like w^{-26D} but has w^- cytology (Figure 2). The recovery of only two wild-type recombinants by crossing over between w^- and fa^{swb} in two experiments already reported (WELSHONS and KEPPY 1975) suggested a map distance of about 13.0×10^{-4}. For this reason, we decided to attempt the reisolation of fa^{swb} and w^- from w^{-26D}, using separate experiments in which selection schemes could be incorporated to increase our chances of recovery of each of the two reciprocal events.

First, we attempted the isolation of fa^{swb} by uncoupling it from w^-. Because w^{-26D} presumably represents the *cis* condition of the two deficiencies, we will symbolize it as $w^-(fa^{swb})$ where the parenthesis indicates that the deletion equivalent to fa^{swb} is present, but the phenotype is suppressed. A crossover between w^- and (fa^{swb}) should yield a fa^{swb} mutant with fa^{swb} cytology. We used three different types of parental females, and sometimes they were mated to different kinds of males because we were attempting to isolate other chromosomes in addition to the fa^{swb} recombinant. For this reason, only the genotypes of the female parents are shown. They were

$$(1)\ \frac{w^-\ (fa^{swb})\ +}{w^a\ N^{55e11}\ rb} \qquad (2)\ \frac{w^-\ (fa^{swb})\ +}{+\ N^{55e11}\ rb} \qquad (3)\ \frac{w^-\ (fa^{swb})\ +\ +}{+\ +\ N^{264\text{-}40}\ rb}$$

in which the symbolism in (1) and (2) reflects the fact that we have not been able to separate fa^{swb} and N^{55e11}. A total of three recombinant chromosomes were detected. One came from cross (2) and two from cross (3); all three had fa^{swb} cytology. There were 982,100 tested chromosomes and the estimated map distance between w^- and fa^{swb} was 6.1×10^{-4}.

To obtain w^- from w^{-26D}, we performed the following cross:

$$\frac{w^- \; (fa^{swb}) \; + \; N^{264\text{-}40} \; rb}{+ \quad + \quad fa^g \quad + \quad +} \times \frac{w^a \; fa^g \; fa^{no}}{Y}$$

(Some $w^a \; fa^g \; fa^{no} \; rb$ parental males were used in a minor fraction of the matings.) Because w^- will not survive as a male, and because w^- and $w^-(fa^{swb})$ can be distinguished only by cytological examination, we screened for recombinant females that were phenotypically dilute w^a (w^-/w^a = dilute w^a) and fa^g. Most of them will have a w^- (fa^{swb}) fa^g $(= w^{-26D} \; fa^g)$ chromosome by crossing over between (fa^{swb}) and fa^g. The event we seek is a crossover between w^- and (fa^{swb}) yielding $w^- + fa^g/w^a \; fa^g \; fa^{no}$ heterozygotes. Among a total of 43 dilute $w^a \; fa^g$ females recovered, only one of the recombinant chromosomes had w^- cytology, representing an exchange event between the deletions. The number of tested chromosomes was equal to 771,400. The estimated map distance w^- to fa^{swb} was 5.2×10^{-4}.

The crossover values obtained in separating w^- from fa^{swb} (5.2 and 6.1×10^{-4}) are about one-half the value of the event that put them together (13×10^{-4}). Perhaps the rate of uncoupling the two is lower than that of coupling, but our estimates are based on only a total of six recombinants and cannot be used to support the view.

In the past, we have commented on salivary band comparisons in our experimental material, and now we are convinced that the following comparisons are valid. Bands 3C1 and 3C7 are virtually equal in density, but in any one cell, they can look relatively light or dense (Figures 2A, B, and E). The heavy bands 3C2,3 and 3C5,6 are about equal, whereas 3C9,10 is definitely lighter than 3C5,6 and, on the average, only slightly heavier than 3C1 or 3C7 (Figures 2A and B). Figure 3C demonstrates the apparent absence of 3C7 in fa^{swb} males, and a 3C5,6 that seems to be slightly larger than 3C2,3. We know the band to the right of 3C5,6 is 3C9,10 and not 3C7 from its pairing behavior in $fa^{swb}/+$ heterozygotes. In spite of cell to cell variations, it seems we can detect differences that are small but reliable.

DISCUSSION AND CONCLUSION

We started with a mutant fa^{swb} that lacked a salivary band where 3C7 ought to be. The heavy band 3C5,6 looked heavier than usual, and we postulated that 3C7 was fused to it. Later, from heterozygotes w^-/fa^{swb}, we isolated a recombinant chromosome that had a heavy band at the 3C1 position, and we presumed that 3C7, fused to 3C5,6 in fa^{swb}, had become fused to 3C1 in w^-; we persisted in this view, even though it required the assumption that fa^{swb} was not expressed when coupled with w^-. Next, we did not simply pick up w^- and fa^{swb} chromosomes out of w^{-26D} and secondarily interpret their origin; we predicted their

regeneration from w^{-26D}, sought and found them. We conclude that small cytological differences should be considered as meaningful conditions worthy of a serious interpretative effort to counter an inclination to cast them off as invalid subjective comparisons or uninterpretable artifacts.

Because fa^{swb} is not expressed when coupled to w^-, and the effect does not seem to be attributable to the formation of a duplication, we feel that fa^{swb} is not a lesion at the Notch locus. The entire gene or locus is present. It is a secondarily induced change in position caused by the deficiency that places Notch in a foreign environment, thereby interfering with normal function and creating the recessive visible "allele" of Notch named fa^{swb}. When the deletion is coupled to the w^- deficiency, the interfering environment is eliminated, and Notch functions are restored. It might be that the interference at Notch is caused by the normal activity of loci to the left of it like the roughest-verticals complex (LEFEVRE and GREEN 1972). Because fa^{swb} is $(rst\text{-}vt)^+$, perhaps we will be able to induce reversions of fa^{swb} simultaneously with the induction of mutants in the neighboring interval. Alternatively, one might view the relatively large mass of DNA in salivary band 3C5,6 as intercalary heterochromatin (HANNAH 1951) capable of inducing something akin to a variegated position effect (LEWIS 1950; BAKER 1968) when the Notch locus is adjacent to it. The crossover event that places Notch next to 3C1 in w^{-26D} might then allow Notch functions to be restored because the quantity and/or activity of band DNA in 3C1 differs from that of 3C5,6. Attempts to couple fa^{swb} to deficiencies other than w^- in the w to N interval might be instructive. At the moment we can only guess as to the nature of adjacent functions interfering with proper Notch function.

It seems that fa^{swb} is not part of Notch; it is a closely linked deficiency that masquerades as a recessive visible allele. It is conceivable that other deficiencies in the same interband 3C5,6 to 7 can completely interfere with Notch function and mimic recessive lethal N alleles when no part of the gene or locus has been deleted. We have five left-side deficiency N mutants with a deletion immediately to the left of 3C7 that extend to, but do not include, 3C1; one, called N^{68f19}, has been used repeatedly in our experiments and serves as an example (WELSHONS 1974; WELSHONS and KEPPY 1975). The mutant condition of N^{68f19} could be a secondary effect on an intact gene caused by the deletion adjacent to 3C7, but, in this case, moving the intact locus to a position adjacent to 3C1 has not suppressed the recessive lethal N, although a similar movement exemplified by the synthesis of w^{-26D} did suppress fa^{swb}. Coupling N^{68f19} to some other deficiency might suppress the mutant effect if N^{68f19} has an intact Notch locus.

The analysis of the right-side deficiency N^{62b1} placed most of the genetic map in the interband 3C7 to 8, and since there was sufficient crossing over in the interband to account for all the recombination expected to be associated with a band like 3C7 (LEFEVRE 1971), it was suggested that exchanges within the band might make only a limited contribution to genetic length (WELSHONS 1974). Chromomeres might facilitate exchanges but not be directly involved. Perhaps that is why the deficiency in fa^{swb} failed to inhibit exchange at Notch. The deletion is mainly interband, the chromosomes are essentially intact, the

facilitation to genetic exchange is normal. Ordinarily, one could expect heterozygosity for a deletion to inhibit recombination in the adjacent region (LEFEVRE and MOORE 1968).

If we assume no crossing over within a chromomere, if the genetic map is mainly in the interband to the right of 3C7, then, because fa^{swb} is an interband deletion to the left of 3C7 and seems to be inseparable from N^{55e11}, this recessive lethal site could also be to the left of 3C7 or within it. Left-side deficiencies like N^{68f19} might be recessive lethal mutants because their deficiencies extend to N^{55e11} at the left margin of 3C7, and right side deficiencies would be lethal because part of the genic map was deleted. The bilateral association of *N* mutants with 3C7 (BEERMANN 1972; WELSHONS 1974) could be explained this way, but the view yields no insight pertaining to the function of DNA composing the salivary band. If, however, there is a bit of truth in this explanation, the left-side deficiency N^{66i25} (WELSHONS 1974; WELSHONS and KEPPY 1975) might be a double mutant. Cytologically, it looks like other *N* mutants with a deficiency to the left of 3C7, but genetically it seems to lack a large piece of the genic map to the right of 3C7. We will attempt to test the hypothesized double mutant nature of N^{66i25}.

The authors wish to express their gratitude for the technical assistance rendered by JEAN WELSHONS.

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