A REVISION OF THE CYTOLOGY AND ONTOGENY OF
SEVERAL DEFICIENCIES IN THE 3A1-3C6 REGION OF THE
X CHROMOSOME OF *DROSOPHILA MELANOGASTER*¹

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ABSTRACT

The cytology and developmental attributes of 18 deficiency mutations in the 3A1-3C6 region of the salivary gland X chromosome of *Drosophila melanogaster* have been investigated. The cytological limits of several older deficiencies have been revised and clarified and several new deficiencies are characterized. The deficiency mutants, with one possible exception, show a lethal phase in the late embryonic period or the early first larval instar. In contrast, the earliest acting point mutation lethals exposed by these deficiencies generally exhibit a somewhat later, post-embryonic lethality, perhaps indicating that the deficiencies are having some cumulative or synergistic impact on development. However, even with this difference in time of lethality, it is still possible to conclude that it is not the absolute size of the deficiency but rather the character of the loci deleted that determines the impact on development. Observations on the morphology of lethal embryos shows that while this analysis is internally consistent, it does not agree with earlier work. None of the 3A1-3C6 deficiencies causes any major teratologies during embryogenesis. Furthermore, the "earliest acting" gene in this region does not lie in band 3C1 but is most likely associated with bands 3A8-10.

THE cytogenetic and developmental analysis of the zeste-white interval, polytene chromosome bands 3A1-3C2, of the X chromosome of *Drosophila melanogaster* (JUDD, SHEN and KAUFMAN 1972; SHANNON *et al.* 1972a,b) revealed that there were some discrepancies between our results and those of previous authors. Our revision of BRIDGES' (1938) original description of this region, described in JUDD, SHEN and KAUFMAN (1972), will be used as a basic framework in this report. Our re-examination of several pre-existing deficiencies in the 3A1-3C6 region shows that the original cytology (SLIZYNSKA 1938) is rather good, with a single exception. We have been able, however, to define more accurately the breakpoints and, therefore, the amount of material missing in

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these deficiencies as well as in several new ones. A developmental analysis of several of these deficiencies is in disagreement with the earlier work of POULSON (1940, 1945). Because POULSON's results have been used as a classical example of gene localization of a lethal effect (HADORN 1955; WRIGHT 1970) and because the present results are internally consistent and in agreement with the *zw* point mutation analysis (SHANNON *et al.* 1972a,b), we feel that a revision of the developmental genetics of these deficiencies is in order.

MATERIALS AND METHODS

A complete description of the mutants used may be found in LINDSLEY and GRELL (1968). Mutants induced or recovered after 1968 or those of special interest are described in the text. All stocks were maintained on standard cornmeal, brewer's yeast, molasses, agar medium. Crosses used to obtain larvae for salivary gland chromosome preparations were carried out at 18°; other crosses were carried out at 25±1°.

Cytology: Both heterozygous female and hemizygous male deficiency stocks have been examined with chromosomes from male stocks proving to be superior for the determination of breakpoints. Male stocks carrying the deficient chromosomes were maintained by balancing to the *w*⁺*Y* chromosome and mating to *C(1)DX/w*⁺*Y* females. Fertilized females in these stocks were allowed to lay eggs on yeasted food for a period of three days at 18°, after which the parents were removed. The larvae from these matings were fed a suspension of live yeast every 48 hours until late third instar larvae were present. The larvae were then removed from the vial, 2 or 3 at a time, and placed in Ringer's solution to be sexed. Male larvae (*Df/w*⁺*Y*) were then transferred to 45% acetic acid and dissected. The salivary glands were removed and transferred to a drop of lacto-aceto-orcein stain on a siliconized slide, where they were allowed to remain for 5–10 seconds. The glands were then immediately squashed in the stain and the coverslip sealed with clear nail polish. This methodology gave excellent resolution of the fine banding and permitted refinement of the various breakpoints.

The genetic extent of all the deficiencies was determined by complementation analysis as described in JUDD, SHEN and KAUFMAN (1972).

Developmental analysis: We have determined the lethal phase and the gross morphology of 18 deficiency mutants in the 3A1–3C6 region that were also examined cytologically. To gather developmental data, we have used the same mating scheme whereby we obtained lethal *zw* point mutation animals in a previous study (see Figure 1 in SHANNON *et al.* 1972b). That is, male deficiency zygotes were selected from the mating γ *Df/Ore-R* ♀♀ × *Ore-R* ♂♂. Some of the deficiency stocks carried various other sex-linked genetic markers; the genotype of all deficiency stocks is indicated in Table 1. Techniques for egg collection and evaluation of embryonic lethality were the same as described in SHANNON *et al.* (1972b).

The gross morphology of live zygotes, prepared as late embryo wholemounts, was examined in all of the deficiency stocks. Unhatched embryos about 24 to 30 hours old were collected, dechorionated manually, mounted in Ringer's solution in shallow depression slides, and examined microscopically. Wholemounts prepared in this manner permitted evaluation of certain dynamic features of the embryos, such as muscular movement and the filling of the tracheal tubes with air. At least 50 embryos per deficiency were studied as wholemounts.

Eight of the deficiency stocks (*w*²⁵⁸⁻¹¹, *w*^{rJ1}, *64c4*, *w*^{rJ2}, *62g18*, *65j26*, *64j4* and the double deficiency *64j4, w*²⁵⁸⁻⁴⁵) were examined histologically. Most histological preparations were made from mutant embryos collected 2–8 hours after normally developing (non-mutant) first instar larvae had hatched. In a few cases, all offspring produced by the mating (unselected *F*₁ sample) were fixed at about the 16th hour of embryonic development, or about 6–8 hours before the normal progeny began to hatch. At this stage of development, egg hatch data could not be relied upon to identify lethal individuals. This procedure, therefore, made possible a blind comparison of normal and deficiency embryos, facilitating detection of possible morphological patterns of damage among lethal zygotes. Eggs were fixed in a 3:1 solution (absolute ethanol

TABLE 1

Cytological extent of deficiencies in the 3A-3C region

Genotype of deficiency	Cytology	Number of bands missing	Origin	Discoverer
γ <i>sc Df(1)X10</i>	Df(1)2F6-3A1;3C3	16-18	X-ray	FALK
γ^2 <i>Df(1)w^{rJ1} spl sn³</i>	Df(1)3A2;3C2	14-16	Recomb.	JUDD
γ <i>Df(1)w²⁵⁸⁻¹¹</i>	Df(1)3A3;3C3	14-16	X-ray	DEMEREK
γ <i>sc⁵ Df(1)w²⁵⁸⁻⁴² spl</i>	Df(1)3A6;3C5	13-15	X-ray	DEMEREK
<i>Df(1)64c4</i>	Df(1)3A3;3C2	13-15	X-ray	JUDD
γ <i>sc Df(1)X12</i>	Df(1)2F6-3A1;3B5	13-15	X-ray	FALK
<i>Df(1)w^{rJ2} ec</i>	Df(1)3A9-10;3C2	8-9	Recomb.	JUDD
γ <i>Df(1)64j4 & Df(1)w²⁵⁸⁻⁴⁵</i>	Df(1)3A9-10;3B1 & Df(1)3B3;3C2	7-9	X-ray	DEMEREK
<i>In(1)w^{-61d8}</i>	Df(1)3B2;3C2 & In(1)3B1-2;20F	6-7	X-ray	LEFEVRE
γ^2 <i>Df(1)w²⁵⁸⁻⁴⁵</i>	Df(1)3B3;3C2	5-6	X-ray	DEMEREK
γ^2 <i>Df(1)K95 cv</i>	Df(1)3A4;3B1	6-7	X-ray	FALK
γ <i>Df(1)w^{67e8}</i>	Df(1)3C2;3C6	2-4	Spont.	GREEN
<i>sc z Df(1)w^{zh}</i>	Df(1)3C2;3C6	2-4	Spont.	JUDD
<i>Df(1)64f1</i>	Df(1)3B1;3B3	3-4	X-ray	ABRAHAMSON
γ <i>Df(1)62g18</i>	Df(1)3A1-2;3A4	3-4	X-ray	JUDD
γ <i>Df(1)65j26</i>	Df(1)3A1-2;3A3-4	2-4	X-ray	ALEXANDER
γ <i>Df(1)64j4</i>	Df(1)3A9;3B1	2-3	Spont.?	DEMEREK?
γ <i>In(1)w^{m1L} rst^{3R}</i>	Df(1)3C2;3 & In(1)3C1-2; 20A ^L 3C3-4;20B ^R	1-2	Synthetic	

to acetic acid), sectioned at 5 μ , stained in aqueous safranin and counterstained with alcoholic fast green. Twenty to 50 embryos per deficiency were examined histologically.

Seven of the deficiencies (*w²⁵⁸⁻¹¹*, *w^{rJ1}*, *w²⁵⁸⁻⁴⁵*, *w^{67e8}*, *62g18*, *65j26* and *64j4*) were also mated to the *X^{C2}*, *w^{vc}* unstable ring X chromosome (HINTON 1955; 1959) to determine if the lethality caused by the deficiency chromosome is autonomous. Males marked with the gene yellow (γ) were mated to *X^{C2}/In(1) dl 49*, γ *l w lz* females and the resultant adult *X^{C2}/γ Df* progeny were scored for the presence of yellow sectors in the integument.

RESULTS

Cytogenetics: The cytological and genetic extent of all of the deficiencies analyzed is summarized in Table 1 and illustrated in Figure 1. Figure 2 shows several of the larger deficiencies and their relative sizes. The following is a brief description of the cytology and origin of the various deficiencies.

The largest of the deficiencies examined is *Df(1)X10*. This mutation was induced with X-rays in the laboratory of DR. R. FALK. The left breakpoint lies to the left of band 3A1 and to the right of band 2F4-5 with the presence of band 2F6 still in doubt. The right breakpoint is between bands 3C3 and 3C5. The resultant chromosome, therefore, brings the 2F5,6 region adjacent to the 3C5,6 region with the subsequent deletion of the 16 to 18 intervening bands (Figure 2).

The next largest deficiency is *Df(1)w^{rJ1}*. This chromosome was the product

FIGURE 1.—Diagrammatic representation of the 3A1-3C6 region of the salivary gland *X* chromosome. Deficiencies are represented by open bars and are shown with respect to the complementation groups which they fail to cover. A stippled area indicates that the corresponding chromomeres are not deficient but that the deficiency chromosome fails to complement the corresponding functional unit. The dashed line at the end of *Df(1)X10* and *Df(1)X12* indicates uncertainty as to left breakpoint. The * indicates no name for function localized to this chromomere.

of regularly occurring unequal exchange between the 3A1,2 and 3C2,3 regions of the salivary gland *X* chromosome (JUDD 1961a,b). It was used as the screen by which a majority of the *zw* lethals were recovered (JUDD, SHEN and KAUFMAN 1972). Cytological analysis reveals that this chromosome is deficient for bands 3A2 through 3C2 inclusive, giving a total of 14 to 16 missing bands (Figure 2).

The third deficiency, *Df(1)w*²⁵⁸⁻¹¹, resembles *w*^{rJ1} in size (ie., number of

TABLE 2

Egg hatch of matings segregating for deficiencies in the 3A-3C region
 The proportion of unhatched eggs in the Oregon-R control stock was 5.6% (22/393).

Deficiency	Number of eggs	Number unhatched	Percent unhatched	Lethal phase
<i>Df(1)X10</i>	770	221	28.7	E
<i>Df(1)w^{rj1}</i>	833	230	27.6	E
<i>Df(1)w²⁵⁸⁻¹¹</i>	239	59	24.7	E
<i>Df(1)w²⁵⁸⁻⁴²</i>	280	79	28.2	E
<i>Df(1)64c4</i>	546	136	24.9	E
<i>Df(1)X12</i>	512	144	28.1	E
<i>Df(1)w^{rj2}</i>	666	149	22.4	E
<i>Df(1)w²⁵⁸⁻⁴⁵ &</i>				
<i>D(1)64j4</i>	352	84	23.9	E
<i>In(1)w^{64d8}</i>	683	72	10.5	E/L
<i>Df(1)w²⁵⁸⁻⁴⁵</i>	789	49	6.2	E/L
<i>Df(1)K95</i>	683	164	24.0	E
<i>Df(1)w^{67es}</i>	629	141	22.4	E/L
<i>Df(1)w^{zh}</i>	463	131	28.3	E
<i>Df(1)64f1</i>	1323	47	3.6	L
<i>Df(1)62g18</i>	661	163	24.7	E
<i>Df(1)65j26</i>	367	86	23.4	E
<i>Df(1)64j4</i>	981	175	17.8	E/L
<i>In(1)w^{m4L} rst^{SR}</i>	550	146	26.5	E

E = late embryo

E/L = late embryo or early first instar larva

L = larva

bands missing) but is shifted to the right (proximally). *Df(1)w²⁵⁸⁻¹¹* was X-ray-induced by DEMEREC and was analyzed cytologically by SLYZINSKA (1938). We have found her original cytology to be essentially correct with the following refinements. The left breakpoint is between bands 3A2 and 3, and the right breakpoint is between bands 3C3 and 5. Therefore, the material from bands 3A3 to 3C5 inclusive is missing, yielding a deletion of 14 to 16 chromomeres (Figure 2).

Another deficiency, *Df(1)w²⁵⁸⁻⁴²*, was induced by DEMEREC with X-rays (LINDSLEY and GRELL 1968). It was supplied to us by DR. E. B. LEWIS. Again, our cytological analysis is in close accord with the original. We have, however, been able to locate the breakpoints more precisely. The left breakpoint is between bands 3A4 and 3A6 (on our map, Figure 1) and the right breakpoint is between bands 3C5 and 3C6. The resultant chromosome lacks 13 to 15 bands, with bands 3A6 through 3C5 inclusive being deleted (Figure 1).

Df(1)64c4 was recovered by JUBB in an experiment using *w^{rj1}* as a screen for lethal mutations. The left breakpoint lies between bands 3A2 and 3A3 and the right breakpoint lies between 3C2 and 3C3. Consequently, bands 3A3 to 3C2 inclusive are missing, creating a 13 to 15 chromomere deficiency (Figure 2).

Df(1)X12 was induced by X-rays and supplied by DR. R. FALK. The distal breakpoint lies to the left of band 3A1 and to the right of band 2F5; the status

of band 2F6 remains in doubt. The proximal breakpoint lies just to the left of band 3C1; therefore, all of sections 3A and 3B are deleted, but all of 3C is intact. This deficiency results in a chromosome lacking 13 to 15 chromomeres.

$Df(1)w^{rj2}$ is, like w^{rj1} , a product of regularly occurring unequal exchange between the fine bands following the 3A1,4 region and the 3C2,3 doublet. The left breakpoint lies to the left of band 3A9 and the right breakpoint lies to the right of band 3C2. The resultant chromosome is deficient for bands 3A9 through 3C2 inclusive and is, therefore, missing 8 to 9 chromomeres (Figure 2).

The next mutation, $Df(1)w^{258-45}$, deserves special attention, since historically there has been much confusion about its cytology. GERSH (1967) stated that w^{258-45} consisted solely of a deficiency for bands 3C1 and 3C2. Later, LEFEVRE and WILKINS (1966) stated that this deficiency lacked bands 3B4,5 through band 3C2. We have obtained stocks of w^{258-45} from several different sources and have found that in all cases it actually consisted of two separate deficiencies which have been separated by recombination in our laboratory. The more proximal and larger of the two deficiencies is more properly described by the cytology of LEFEVRE and WILKINS; i.e., the left breakpoint is to the right of the 3B1,2 doublet and the right breakpoint is to the right of band 3C2. Therefore, bands 3B3 to 3C2 inclusive, 5 to 6 bands, are missing (Figure 2). The distal and smaller portion of this double deficiency could have been induced simultaneously with the induction of the proximal portion (DEMEREK 1938, cited in LINDSLEY and GRELL 1968), or it could have arisen spontaneously at a later date. We favor the latter explanation. We have named the distal segment $Df(1)64j4$; this deficiency lacks only two or three chromomeres, band 3A9 and 10 and band 3B1. The double deficiency is, therefore deficient for bands 3A9–3B1 plus bands 3B3–3C2 inclusive, a total of 7 to 9 bands. Band 3B2 is apparently intact. We propose that the proximal portion retain the original designation $Df(1)w^{258-45}$ and that the distal segment be called $Df(1)64j4$. The double deficiency should then be indicated as $Df(1)64j4, Df(1)w^{258-45}$ (Table 1).

$In(1)w^{64ds}$ is a combination X-chromosome inversion and deficiency. This chromosome was supplied to us by Dr. G. LEFEVRE and our cytology is in agreement with his (LEFEVRE 1968b). The left breakpoint of the deficiency lies between bands 3B1 and 3B2, and the right breakpoint lies between bands 3C2 and 3C3, thus yielding a 6 to 7 band deficiency. Bands 3B2 to 3C2 inclusive are missing.

Another X-ray-induced deficiency, $Df(1)K95$, was supplied to us by Dr. R. FALK. The left breakpoint is between bands 3A3 and 3A4 (just to the right of, but not including, the zeste locus) and the right breakpoint is between bands 3B1 and 3B2. The resultant chromosome has a diffuse band (3B2) immediately

FIGURE 2.—Photomicrographs of selected 3A1–3C6 deficiency chromosomes. The chromosomes are arranged in roughly descending order with respect to size, with the smallest at the top and the largest at the bottom. Lines above the Oregon-R chromosome indicate, from left to right, the beginning of sections 3A, B and C and the end of section 3C. Below the Oregon-R and between the deficiency chromosomes, the lines from left to right indicate the beginning of section 3A, section 3B and the proximal edge of 3C7.

adjacent to a dark staining band (3A3), giving a 6-7 band deficiency lacking bands 3A4 to 3B1 inclusive (Figure 2).

The next three deficiencies are located in the 3C2 to 3C6 region. Both *Df(1)w^{ses}* and *Df(1)w^{zh}* are identical in size and spontaneous in origin; the former was discovered by GREEN (1967) and the latter by JUDD. Both lack bands 3C2 through 3C6 inclusive and are, therefore, deficient for 4 bands (2 doublets on our map). The other deficiency in the 3C region is *In(1)w^{m4L}rst^{3R}*, a synthetic lethal constructed by recombination. It was kindly supplied by Dr. GEORGE LEFEVRE. The mutant chromosome is deficient for bands 3C2 and 3C3 but not for bands 3C5 and 3C6. According to LEFEVRE (1968a), a deficiency of this extent should not cause lethality. Because of the inversion of the *X* chromosome, however, there is a position effect "deletion" of bands 3C5 and 3C6 and a resultant functional loss of the material from band 3C2 to band 3C6 inclusive (LEFEVRE and GREEN 1972).

Two additional deficiencies involve the 3A1 to 3A4 region of the *X* chromosome. *Df(1)62g18*, which was X-ray-induced, was recovered in the screen for the *zw* mutations (JUDD, SHEN and KAUFMAN 1972). One thin dark band of the original 3A1.4 group remains. The 2F region and the remainder of the 3A region (3A6-10 on our map) appear to be intact. Three chromomeres, therefore, are missing, probably bands 3A2.3 and 4, while band 3A1 in all likelihood contains a mutation or is only partially deficient.

The cytology of the second deficiency, *Df(1)65j26*, is somewhat puzzling when its genetics is considered. The complementation patterns of *65j26* and *62g18* are identical (JUDD, SHEN and KAUFMAN 1972). *Df(1)65j26*, however, lacks only two chromomeres in the 3A1.4 region; band 2F6 to the left and band 3A6 to the right are unaffected. We believe the most likely explanation for these facts is that *65j26* lacks bands 3A2 and 3A3 and that bands 3A1 and 3A4 are mutated or partially deficient. However, it is also possible that a thin band exists in this region which has not been resolved by our cytological observations. It should be possible to show if bands 3A2 and 3A3 are deficient by the cytological examination of heterozygous female larvae. Unfortunately, however, this particular region of the chromosome does not lend itself well to cytological analysis in the heterozygous condition, as the bands tend to clump together.

The final deficiency, *Df(1)64f1*, was X-ray-induced and supplied to us by Dr. S. ABRAHAMSON. The left breakpoint lies between bands 3A10 and 3B1 and the right breakpoint lies between bands 3B3 and 3B4. Thus, the 3B1.2 doublet and one additional fine band, 3B3, are missing.

Development: All 18 mutants examined seem to undergo a normal or almost normal embryogenesis; i.e., there is no consistent evidence of teratologies during the embryonic period, yet most die at the end of the embryonic period (Table 2). In the case of at least four mutants, *64j4*, *w²⁵⁸⁻¹⁵*, *w^{-64ds}* and *w^{67es}*, some larvae actually hatch but do not grow. Egg hatch values in matings segregating for some of the mutants which we classified as late embryonic lethals are slightly less than 25%, the fraction of the zygotes expected to carry the deficiency chromosome in the male. Considering that some eggs are usually unfertilized

(about 6% in the Oregon-R stock), it seems likely that these mutants also occasionally hatch. *Df(1)64f1* is a post-embryonic, or larval, lethal; our observations show that *64f1* larvae die before puparium formation, but we have not precisely established the lethal phase.

Mutant individuals prepared as late embryo whole-mounts generally had either normal or almost normal gross morphology (Figure 3). Mutant embryos underwent regular, co-ordinated muscular contractions, although movement was usually less vigorous than in normal individuals. In mutant individuals, the gut was usually highly convoluted and exhibited vigorous peristalsis. We observed slight tracheal irregularities (local pinches or breaks in the main trunks and apparent lack of uniform thickness) in some mutant embryos and also in Oregon-R control embryos. We feel that these irregularities might have been artifacts induced by handling the material. We observed some embryos of each mutant genotype with structurally normal trachea. In most mutant embryos examined, the trachea became completely or partially gas-filled; however, no gas was observed in the trachea of *X10*, *w^{67es}* and *w^{zh}* embryos. In mutant individuals with incompletely gas-filled trachea, the posterior regions generally contained gas, but the anterior regions did not. The mutants *64c4* and *62g18* were peculiar in that usually only the anterior part of the tracheal system became gas-filled (Figure 3E,F); this phenomenon was also observed in a few *X12*, *w²⁵⁸⁻¹¹*, *K95* and *w^{m4L} rst^{SR}* embryos. This pattern of gas uptake was different from that characteristic of normal embryos, in which gas enters the trachea in a posterior to anterior sequence (KALISS 1939). At the time normal (non-mutant) first instar larvae were hatching, many mutant embryos had a relatively large amount of yolk in the gut (Figure 3A,H) and the Malpighian tubules contained opaque excretory products (Figure 3B,C,H). The moribund mutant embryos continued to accumulate material in the Malpighian tubules, which appeared thick and distended about a day after normal larvae had hatched (Figure 3D,F). As judged by muscular movement, unhatched mutant embryos often lived for as long as 48 hours after egg deposition (Figure 3F).

In addition to the relatively minor defects described above, gross morphological defects were sometimes noted in certain of the mutants (Figure 4). Teratologies occurred in roughly 10% of both *64j4*, *w²⁵⁸⁻⁴⁵* and *w²⁵⁸⁻⁴⁵* embryos and in about 5% of *w²⁵⁸⁻¹¹* embryos. In the other mutants, severe morphological defects were rare or absent. There was no distinct syndrome among the abnormal embryos. The more highly developed ones tended to have imperfect segmentation, defects of the cephalopharyngeal apparatus and incomplete gut convulsion; intestinal peristalsis occurred, but there was little or no movement of the somatic musculature (Figure 4D). Some abnormal embryos had hernias of the yolk mass or of the gut. Others were amorphous in appearance, although weak twitching movements sometimes occurred (Figure 4C).

The eight mutants examined histologically were generally indistinguishable from normal (non-mutant) embryos (Figure 5). Occasionally, however, a mutant embryo had an incompletely condensed ventral nervous system and/or more than the normal amount of yolk in the gut.

TABLE 3

Results of cellular autonomy experiments with selected 3A-3C deficiencies

Genotype of rod X	Number of Xc2/deficiency heterozygous females	Number of gynandromorphs	
		Observed	Expected
<i>In(1)d149 γ l w lz</i>	402	143	Control
<i>γ Df(1)w^{rj1} spl sn³</i>	219	0	78
<i>γ Df(1)w²⁵⁸⁻¹¹</i>	228	0	81
<i>γ Df(1)w²⁵⁸⁻⁴⁵</i>	193	0	68
<i>γ Df(1)62g18</i>	129	0	46
<i>γ Df(1)65j26</i>	176	0	62
<i>γ Df(1)64j4</i>	110	0	39
<i>γ Df(1)w^{67e8}</i>	103	13	36

The results of the ring X study are given in Table 3. None of the deficiency stocks except *w^{67e8}* produced gynandromorphs. Those produced by crosses involving *w^{67e8}* showed a definite mutant phenotype. When the mutant tissue was in the eye, it was rough and lacked pigment. In the dorsal region of the head, varying numbers of ocellar and orbital bristles were missing, most often the posterior verticals. In the thorax, the dorsocentral and alar bristles were often missing and the wings, when yellow, were wrinkled and misshapen. These phenotypic changes are not surprising since this chromosome is deficient for the *w*, *rst* and *vt* loci (Figure 1).

DISCUSSION

Cytology: Our revision of BRIDGES' cytology of the 3A1-3C6 interval entails the removal of one band, 3A5, and the addition of at least one band to section 3B (3B5). These changes are based solely on structures visible to us with the light microscope using wild-type chromosomes. Figure 1 does not, therefore,

FIGURE 3.—Whole-mount preparations of certain 3A-3C deficiency embryos. The photographs show the typical appearance of mutant individuals late in the embryonic period. See Figure 4 for comparison with a normal Oregon-R embryo. The embryos were 24-30 hours old and actively moving unless otherwise noted; anterior ends are at left. X150. Abbreviations: cpa, cephalopharyngeal apparatus; mg, midgut; mh, mouth hook; mp, Malpighian tubules; ps, posterior spiracle; tr, trachea; vit, vitalline membrane; y, yolk.

- A. *Df(1)X10*. Ventral view. Note the relatively large amount of yolk.
- B. *Df(1)w^{rj1}*. Dorsolateral view.
- C. *Df(1)w²⁵⁸⁻¹¹*. Lateral view.
- D. *Df(1)w²⁵⁸⁻¹¹*. 48 hours old. Dorsal view. This embryo did not exhibit movement and was apparently dead. Note the large accumulation of materials in the Malpighian tubules.
- E. *Df(1)64c4*. Dorsal view. Note that only the anterior portions of the main tracheal tubules were filled with air.
- F. *Df(1)64c4*. 48 hours old. Dorsal view. Note the appearance of the trachea and the Malpighian tubules.
- G. *Df(1)w²⁵⁸⁻⁴²*. Dorsal view.
- H. *Df(1)X12*. Ventral view.

FIGURE 4.—Whole-mount preparations of certain abnormal 3A–3C deficiency embryos with a normal Oregon-R embryo for comparison. Embryos were 24–30 hours old; anterior ends are at left except in 4D. X150. Abbreviations: cpa, cephalopharyngeal apparatus; ps, posterior spiracle; tr, trachea; vit, vitelline membrane; y, yolk.

- A. Oregon-R. Dorsal view.
- B. *Df(1)w²⁵⁸⁻¹¹*. Lateral view. Note the large yolk mass.
- C. *Df(1)64j4, Df(1)w²⁵⁸⁻⁴⁵*. Ventral view. Note the amorphous appearance and the large yolk mass.
- D. *Df(1)w²⁵⁸⁻⁴⁵*. Lateral view. Note the distinct posterior segmentation but apparent lack of anterior segmentation. The arrow indicates uninvoluted head material.

reflect findings with the electron microscope (BERENDES 1970; SORSA, GREEN and BEERMANN 1973) or recent findings using mutant chromosomes (SORSA, GREEN and BEERMANN 1973; JUDD 1974). The results of the electron microscope studies have revealed the presence of at least one more fine band in section 3B (3B6) and the possibility that band 3A4 may, in fact, be two bands (BEERMANN 1972). It is also possible to resolve a fine band (3C1.5) between bands 3C1 and the 3C2,3 doublet in certain mutant strains (SORSA, GREEN and BEERMANN 1973; JUDD 1974). These considerations make our estimates, as given in Table 1, of total number of bands missing for the 3A1–3C6 deficiencies tentative. The right and left breakpoints, however, would seem to be fairly definitive. Nonetheless, studies of these deficiency-bearing chromosomes using the electron microscope may provide a final resolution of the absolute size of these deletions.

FIGURE 5.—Histological preparations showing the typical appearance of certain 3A–3C deficiency embryos late in the embryonic period. For comparison with wild-type embryos, see POULSON (1950). Age at fixation was about 20 hours; anterior ends are at left. X240. Abbreviations: br, brain; ch, chorion; cpa, cephalopharyngeal apparatus; hg, hindgut; hy, hypoderm; mg, midgut; mh, mouth hook; pv, proventriculus; slg, salivary gland; sm, somatic muscle; vns, ventral nervous system.

- A. *Df(1)w^{rJ1}*. Sagittal section.
- B. *Df(1)w^{rJ1}*. Frontal section.
- C. *Df(1)w²⁵⁸⁻¹¹*. Frontal section.

None of the above observations seriously impairs the one gene:one band hypothesis (JUDD, SHEN and KAUFMAN 1972). There are, however, certain "functions" which were not screened for in the original zeste-white mutant hunt, such as the "clock" mutant which apparently maps in the 3A1–3C6 interval (KONOPKA and BENZER 1971) and a complex female-sterile locus (JUDD and YOUNG 1973) also localized to this interval. The status of these two "new" types of function, with respect to the lethal complementation map, is currently under investigation and the results of these analyses will be the subject of future reports. There is, therefore, a possibility that the band:gene assignments of

JUDD, SHEN and KAUFMANN (1972) are inexact and should not be taken absolutely, as the band:gene localizations could be shifted, but by no more than one or two chromomeres.

Development: A number of genetic variations in *Drosophila* are known which appear morphologically normal or nearly normal at the end of the embryonic period (IMAIZUMI 1958; WRIGHT 1970). Most of the 3A-3C deficiency mutants we have examined belong to this category. At least four of the 3A-3C mutants can be classified as embryonic-larval "boundary lethals" since some larvae hatch before death occurs (HADORN 1951). The 3A-3C mutants resemble other "physiological" lethals in having reduced muscular activity, opaque Malpighian tubules and defects in the uptake of gas by the trachea. The 3A-3C mutants are also like other late-embryonic *Drosophila* lethals in their capacity for prolonged survival beyond the lethal phase (cf. HADORN 1955; SHANNON *et al.* 1972b; SHANNON 1973).

Our observations do not agree with previous findings of POULSON (1945) who described a syndrome of embryonic abnormalities in the three white-deficient mutants, w^{258-11} , w^{258-14} and w^{258-45} . According to POULSON, these mutants became abnormal between the 12th and 16th hours of embryonic development. Ectodermal derivatives were essentially normal, but the mid-gut remained sac-like and full of yolk. Mesodermal rudiments formed but then degenerated, resulting in an embryo without visceral and somatic musculature. WRIGHT (1970) also maintained that the " w^{258-45} syndrome" characterized three additional white-deficient mutants, w^{258-42} , w^{258-48} and w^{Vogt} . None of the mutants we have studied exhibits the syndrome POULSON described, although three, w^{258-11} , w^{258-42} and w^{258-45} , are among those said to show the syndrome and three others are cytologically similar to presumably affected mutants ($64c4$ resembles w^{258-14} ; w^{rJ2} resembles w^{258-48} ; and w^{64ds} resembles w^{Vogt}).

There is no obvious explanation for the discrepancy between our observations and the earlier work of POULSON. Perhaps the stocks he used contained certain linked but unrelated deleterious genes which were responsible for the abnormalities he observed. This can be easily accounted for by the fact that when the earlier studies were performed, duplications such as w^+Y were not available. Therefore, *X*-linked lethals had to be maintained in females, thereby allowing the accumulation of mutations at other loci on the deficiency-bearing chromosome. Interestingly, the two mutants, w^{258-45} and w^{258-11} , in which we have consistently noted a low frequency of teratologies, are the same as two of the three originally studied by POULSON. If genetic factors that disrupt embryogenesis originally existed in the 258 deficiency series, it is, of course, possible that their expression has changed because of the appearance of modifiers or because of environmental differences (cf. DOANE 1960). We have attempted to reduce the influence of any deleterious genetic factors by use of the w^+Y balancer (thus exposing all but a small part of the *X* chromosome to selection) and by outcrossing the deficient chromosomes to an Oregon-R background. The consistency of our results, which are based upon mutant stocks obtained from several laboratories, would seem to indicate that the developmental pattern we have

found is a genuine attribute of deficiencies in the 3A1–3C6 region of the X chromosome.

Our use of the w^+Y chromosome for the prevention of accumulation of secondary changes has not, however, been foolproof. We noted that in a few stocks some of the deficiency-bearing males had lost the dominant Confluens (*Co*) phenotype. By outcrossing these males, we found that they had acquired a new mutation at the Notch locus. Therefore, accumulation of second-site lethal mutations is possible even in the small section of the X chromosome covered by the duplication but not deleted in the deficiency chromosome.

The present work should help dispel the considerable confusion that exists regarding both the cytological limits of $Df(1)w^{258-45}$ and the nature of the lethality associated with this deficiency (LEFEVRE and WILKINS 1966; GERSH 1967; LEFEVRE 1968a,b). It is now known that a zygote deficient for band 3C2, but not band 3C1, survives as a white-eyed male and that w^{258-45} lacks not only bands 3C2 and 3C1, but several bands to the left of 3C1 (see LEFEVRE and WILKINS 1966; JUDD, SHEN and KAUFMAN 1972). The statement in a current review on the genetics of *Drosophila* embryogenesis (WRIGHT 1970) that w^{258-45} is a two-band deficiency, presumably lacking only bands 3C2 and 3C1, is, therefore, in error. Moreover, the existence of a " w^{258-45} syndrome" attributable to the loss of band 3C1 or to a few nearby bands, is now in doubt. Eight of the mutant chromosomes we have examined are deficient for bands 3C2, 3C1 and a number of additional bands (see Figures 1 and 2); it is simply not possible to attribute the lethality of these mutants to any one band. Furthermore, it seems unlikely that lethal point mutations which map very close to the white locus cause embryonic defects similar to those originally described by POULSON. In point of fact, the mutations which are localized to band 3C1 (JUDD, SHEN and KAUFMAN 1972) do not impair embryogenesis at all but bring about death in the third larval instar or in the pupal period (SHANNON *et al.* 1972b).

LEFEVRE (1968a) noted that male zygotes deficient for bands in the 3C2–3C6 region sometimes survive to the adult stage. In our experience, however, all males deficient for these bands succumb at the end of the embryonic period (see Table 2, data on w^{67es} , w^{2h} , w^{m4L} *rst*^{3R}).

JUDD, SHEN and KAUFMAN (1972) have found a good correlation between the number of cistrons and the number of chromomeres in the 3A1–3C2 region. Lethal and semi-lethal mutants belonging to a given cistron exhibit similar lethality patterns and morphological attributes (KAUFMAN 1970; SHANNON *et al.* 1972b). It would be interesting to know if deficiencies for corresponding single bands would confer the same developmental and morphological characteristics. Unfortunately, none of the deficiencies utilized in the present work lacks only a single band. Mutant males belonging to the *zw2* cistron, localized to bands 3A9–10, do, however, have the same lethal phase as $Df(1)64j4$ males, which lack bands 3A9–10 and 3B1 (Figure 1). Both *zw2* males and $64j4$ males die at the late embryonic-early larval boundary. Mutant males belonging to the *zw3* cistron, localized to 3B1, die primarily during the third larval instar. Significantly, the lethal phase of $64j4$ corresponds to that of the mutant locus characterized by earlier lethality. All other deficiencies we have examined also

cause death as early as or earlier in ontogeny than do the various point mutations exposed by these deficiencies. The point mutations typically cause post-embryonic lethality, whereas the deficiencies bring about late embryonic lethality (see SHANNON *et al.* 1972b). This fact may indicate that the deficiencies exert a cumulative effect upon the lethal phase.

Our results indicate that none of the deficiencies embracing the 3A1–3C6 region interferes in any major way with embryogenesis (i.e., there are no gross morphological anomalies). Certainly it is no longer permissible to maintain that the “earliest acting” gene in this region lies in band 3C1. It would appear that the “earliest acting” genetic unit near this region is the Notch locus, localized in band 3C7 (SLIZYNSKA 1938). None of the mutants discussed in the present work is deficient for this band, and none shows the Notch pattern of damage. We did, however, investigate a new Notch mutation that arose spontaneously in one of our deficiency stocks (see above); males bearing this mutation become abnormal during early embryogenesis and exhibit defects almost identical to those described in *Notch-8* embryos by POULSON (1940, 1945).

The results of the cellular autonomy study were not unexpected. Each of the three large deficiencies (w^{rj1} , w^{258-11} and w^{258-45}) exposes point mutation lethals which are themselves autonomous (SHANNON *et al.* 1972b). Three of the smaller deficiencies (62g18, 65j26 and 64j4) uncover point mutations which are non-autonomous; therefore, one might expect that they too would prove to be non-autonomous. However, even in very large numbers, they gave no gynandromorphs. These results, like those on lethal phases, probably reflect the impact of several mutant genes on the development of deficiency-bearing individuals. The one deficiency which did give several gynandromorphs was w^{67e8} ; it exposes the w locus but otherwise lies to the right of the zw interval. It is likely that we did not see some w^{67e8} gynandromorphic individuals and that we mis-scored others, especially where eye tissue was involved. The ring X chromosome used variegates for the genes w , rst and vt , all of which are absent in the w^{67e8} chromosome. Unless the eye had yellow tissue and bristles around it, therefore, it was not scored as gynandromorphic tissue. We found several individuals which did have yellow tissue and bristles and it is these progeny which are listed in Table 3. In summary, it would appear that deficiencies, even very small ones, in the zw region rarely, if ever, show non-autonomy, whereas small deficiencies to the right of the region may occasionally do so.

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