THE GENETICS OF A SMALL AUTOSOMAL REGION OF *DROSOPHILA MELANOGASTER*, INCLUDING THE STRUCTURAL GENE FOR ALCOHOL DEHYDROGENASE. V. CHARACTERIZATION OF X-RAY-INDUCED *Adh* NULL MUTATIONS

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ABSTRACT

Of 31 X-ray-induced and 2 spontaneous *Adh* null mutations selected for resistance to pentenol (AARON 1979), 21 are deletions, including *Adh* and one or more neighboring loci. By contrast, none of 13 EMS-induced Adh^n mutations are deletions. On average, the size of these X-ray-induced deletions is shorter than that of 12 formaldehyde-induced Adh^n deletions (O'DONNELL, MANDELL, KRAUSS and SOFER 1977). Both the X-ray- and formaldehyde-induced deletions show a nonrandom distribution of break points in region 34D to 35D of chromosome arm 2L. Some of the deletions display particular genetic properties associated with one of their end points.

THE discovery (SOFER and HATKOFF 1972) that the treatment of adult *Drosophila melanogaster* with various unsaturated secondary alcohols could form the basis of a selection screen for the recovery of mutations lacking a functional alcohol dehydrogenase has allowed the isolation of 31 X-ray-induced Adh^n mutations (AARON 1979). In view of the fact that few detailed genetic and molecular analyses of a reasonably large number of X-ray-induced mutations have been done in Drosophila, we have characterized these *Adh* mutations in some considerable detail. It is apparent that the great majority of them are deletions.

The mutant *Adh* alleles were recovered after mating X-rayed *b* Adh^F *cn bw* males to Adh^{fn23} *cn* bw^D/*In(2LR)O, Cy* dp^{lvl} Adh^{nB} *pr* cn^2 females and selecting their progeny for survival to exposure to the vapor of 1-penten-3-ol. All flies with an active ADH die and only those that carry a newly induced Adh^- mutation on their *b* Adh^F *cn bw* chromosome survive. The objective of the original experiments (AARON 1979) was to compare the frequency of Adh^- mutations at different X-ray doses; in addition to the 31 induced mutations, 2 occurred in control experiments, i.e., were spontaneous (one (*nLA319*) arose, not on the *b* Adh^F *cn bw* chromosome, but on Adh^D *pr cn*).

In his initial characterization of these mutations, AARON (1979) distinguished

between those that were viable (either as homozygotes or when heterozygous with a long deletion including *Adh* and several adjacent lethal complementation groups) and those that were homozygous, or hemizygous, lethal. The viable mutations (coded *nLA*) were, in general, regarded as "point" mutations and the lethals (coded *Df(2L)A*) as deletions, including not only *Adh*, but also at least one neighboring lethal complementation group.

AARON noted that certain heterozygous combinations of different mutations survived, but that the flies showed a "peculiar wing effect," the wings being bent downward and warped. As we will show, this is a consequence of these genotypes being homozygously deficient for outspread (*osp*), the locus immediately distal to *Adh*. More detailed study of these heterozygous genotypes showed that some expressed a third phenotype, in addition to being Adh^{-} osp^{-} they lacked the ocelli and associated chaetae. This, we will show, is a result of their being homozygously deficient for a third locus, no-ocelli (*noc*), which maps immediately distal to *osp*.

This paper describes the genetic characterization of the induced mutations. The molecular analysis of these mutations will be published elsewhere (R. KARP, M. BODMER and H. PELHAM, unpublished).

MATERIALS AND METHODS

Stocks: Of the 33 mutations recovered by AARON (1979), three, *(Df(2L)A167, Df(2L)A76* and *Df(2L)A385)*, were lost before these studies began. The remaining 30 are listed in Table 1. Other stocks have either been described before (WOODRUFF and ASHBURNER 1979a, 1979b) or will be described in the text. Standard procedures were used for all the crosses, which were done at 25° on Phillip-Harris "instant" Drosophila medium. Crosses were normally scored until the 18th day after setting up. Unless stated, all viability data are from crosses between mutations balanced against *In(2LR)O,Cy* dp^{lvl} *pr* $cn^{2}(\pm\ Adh^{nB})$ and the data are expressed as the number or fraction of Cy^{+} progeny over total progeny number.

Cytology: Polytene chromosome cytology was done on larvae reared on a yeast-glucose medium at 25°. Breakpoints of aberrations were interpreted with the aid of the revised *2L* map (BRIDGES 1941).

Fertility tests: To evaluate the fertility of particular genotypes five males or five nonvirgin females of the appropriate genotype were mated to five virgin females or five males from a Canton-S stock in vials 1-inch in diameter on yeast-glucose medium. If, after 4–5 days, the cultures were apparently sterile, the flies were transferred to fresh vials. At least five replica crosses were set up per test; in the case of sterile or semisterile cultures, at least ten replicas were set up. Flies were scored as "fertile" if they produced abundant progeny (over 50/vial), as "semifertile" if one or more vials produced few progeny (0–50/vial), as "semisterile" if one or more vials produced fewer than ten progeny and as "sterile" if all vials were nonproductive.

ADH assay: Ten 5-day-old adult flies were homogenized in 1.0 ml of 0.05 M phosphate buffer (pH 7.5), and after centrifugation for 6 min in an Eppendorf Microfuge, 0.1 ml of the supernatant was added to 0.9 ml of assay cocktail (final concentrations—1.9 mM NAD^{+}, 0.13 M butan-2-ol, in 0.05 M sodium phosphate buffer at pH 7.5). The change in absorbance at 340 nm was measured in a spectrophotometer. Extracts were kept at 0–4° until assay at 30°.

RESULTS

Cytology: The polytene chromosomes of all of the mutations have been examined and the data are summarized in Table 1. All of the *Df(2L)A* series are, cytologically, deletions of bands in region 35 of chromosome arm *2L*, and their

TABLE 1

Cytological characteristics of A *and* nLA *series of* Adh *null mutations*

Stock	Irradiation dose (kR)	Cytology
Df(2L)A47	3	*Df(2L)34E1;35B2*
Df(2L)A48	3	*Df(2L)35B2-3;35D5-7*
Df(2L)A63	3	[a]
Df(2L)A72	3	*Df(2L)35B2-3;35B7-8*
Df(2L)A178	3	[a]
Df(2L)A215	1	*Df(2L)35A3;35C1-3*
Df(2L)A217	1	*Df(2L)34F5;35B3*
Df(2L)A220	0.5	*Df(2L)35B1-2;35B9*
Df(2L)A245	0.5	*Df(2L)35A4;35B2*
Df(2L)A246	0.5	*Df(2L)34F4;35D3-4*
Df(2L)A260	0	*Df(2L)35B1-2*
Df(2L)A263	1 (?)	*Df(2L)34E5-F1;35C3-5*
Df(2L)A264	1	*Df(2L)35B1-3;35B8-9*
Df(2L)A266	1	*Df(2L)35B2-3*
Df(2L)A267	1	*Df(2L)35B2;35B10*
Df(2L)A376	3	*Df(2L)34E3;35C4-5*
Df(2L)A377	3	*Df(2L)34F1-4;35F1-2*
Df(2L)A379	3	[a]
Df(2L)A400	3	*Df(2L)35A1-4;35B10*[a]
Df(2L)A445	3	*Df(2L)35B1-2*
Df(2L)A446	3	*Df(2L)35B1-2;35E6*[a]
nLA2	1	Normal
nLA73	3	Normal
nLA74	3	Normal
nLA80	3	Normal
nLA248	0.5	Normal
nLA249	0.5	Normal
nLA252	0.5	Normal
nLA319	0	Normal
nLA378	3	Normal
nLA405	3	Normal

[a] See text: cytologically complex.

common region confirms the location of *Adh* to 35B2-3 (WOODRUFF and ASHBURNER 1979a). Several of the mutations were, cytologically, rather complex and these will now be described.

nLA63: Genetically *nLA63* is a small deletion, in addition to being Adh^- it is also osp^- (see below). Cytologically this deletion is not evident. The original *nLA63* chromosome was associated with two independent translocations between chromosomes 2 and 3, i.e., *T(2;3)CA1* (= *T(2;3)45D1-2;96D10-11*) and *T(2;3)CA2* (= *T(2;3)42D6;67A3-4*). These translocations severely reduced the viability of the *nLA63* stock and all of the genetic experiments used a translocation-free *b nLA63* rd^s *pr cn* chromosome derived by exchange between *nLA63 b cn bw,T(2;3)CA1 T(2;3)CA2* and *b* el^1 rd^s *pr cn*.

nLA178: This mutation proved to be a deletion, by genetic analysis, for *noc*, *osp* and *Adh*. Cytologically it is not an obvious deletion, however, on occasion, the loss of 35B2-3 or even 35B1-3 can be seen. It, too, carried an independent

translocation, *T(2;3)CA3* = *(T(2;3)41F;90AB)* that was removed by exchange with *b el^{l} rd^{s} pr cn* before the mutation was used in genetic experiments.

nLA379: Genetically *nLA379* is, like *nLA178*, deleted for *noc*, *osp* and *Adh*. This deletion is associated with a complex inversion on chromosome *2 (In(2LR)A379)* with the new order: *21—35B3-5|57A8-10—41|35B3-5—41|57A8-10—60*, *i.e.*, *In(2LR)35B3-5;57A8-10* + *In(2)35B3-5;41*. It has not been established that the region *41* break is indeed on *2R*, rather than on *2L*.

A400: In addition to *Df(2L)35A1-4;35B10* the *A400* mutation carries a long insertional translocation of region *89A* to 94F into *2R* at *50EF* (order: *21—50EF|94F—89A|50EF—60*) (= *T(3;2)CA4*) and a complex translocation between chromosome arm *3R* and *4* plus an inversion on *3R* (*T(3;4)CA5* + *In(3R)CA6*) so that the new order of chromosome *3* is: *100—94F|101.;61—80-81|89A—80-81|101-102*.

A446: The final complex mutation is *A446*. This chromosome has suffered four breakage events. In effect, the region between 35B1 and 36C1-2 was "removed" from *2L*, shortened by a third break at 35F1-2, the 35B1-35E6 region being lost and the 35F1-2 to 36C1-2 region being inserted into *2R* at 49B1.7 with the order *21—35B1-3|36C1-2—49B1-3|35F1-2—36C1-2|49B4-60*. By exchange between this transposition and a structurally normal chromosome the *Dp(2;2)A446* = *Dp(2;2)35F1-2;36C1-2* is easily recovered. The reciprocal deletion *Df(2L)35B1-2;36C1-2* is very weak, appears to be sterile and have a dominant wavy wing and small fly phenotype.

Deletion mapping of the Df(2L)A *series*: Between bands 34D4 and 35E1-2, 28 lethal and 9 "visible" complementation loci have been mapped (O'DONNELL *et al.* 1977; WOODRUFF and ASHBURNER 1979b; M. ASHBURNER and colleagues, unpublished). All of the available *Df(2L)A* deficiencies have been crossed to representatives of these loci, and the data not only confirm that all of these mutations are deletions but also map their limits. The data are summarized in Figure 1.

Figure 1 is a revised version of the map published by WOODRUFF and ASHBURNER (1979b). It includes new information from the present experiments and from other, unrelated experiments. The revisions to the map include several new complementation groups (*i.e.*, *l(2)br28* to *l(2)br37*), one new "visible" locus (*noc*, see below) and more detailed mapping of several loci. Full justification for those revisions not evident from the mapping of the *Df(2L)A* deficiencies will be published at a later date. We point out that the orientation of *Adh* and *osp* is different from that previously reported (WOODRUFF and ASHBURNER 1979b). The orientation shown in Figure 1 is supported by very much more extensive recombination data than were previously available (C. DETWILER and M. ASHBURNER, unpublished) and by the recovery of two *noc^{-} osp^{-} Adh^{+}* deletions (*Df(2L)osp144*, and *Df(2L)TE146-GH*) (C. DETWILER and M. ASHBURNER, unpublished).

AARON (1979) noted that certain heterozygous combinations of different deletions were viable but that the flies had a warped wing phenotype. E. H. GRELL (personal communication) discovered a recessive mutation, outspread-wings (*osp*) within *Df(2L)64j* and this has been mapped immediately distal to

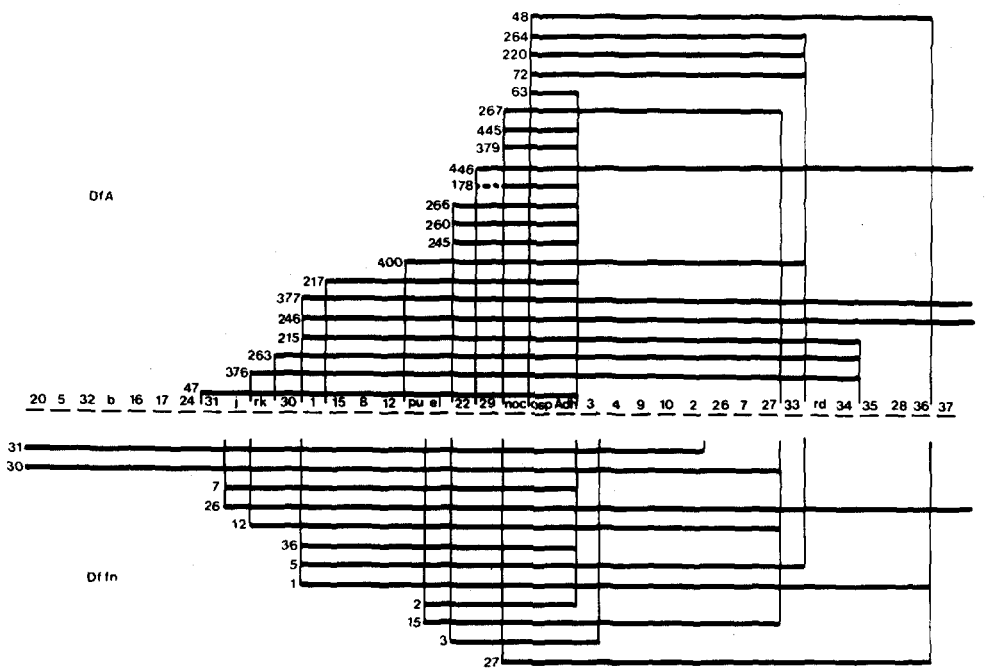

FIGURE 1.—A genetic map of region 34D1.2 to 35E1.2 showing the genetic limits of the X-ray-induced *Df(2L)A* deficiencies and, for comparison, those of the formaldehyde-induced *Df(2L)fn* deficiencies (see DISCUSSION). Lethal loci (*l(2)br1* to *l(2)br37*) are indicated only by their numbers. Non-lethal loci are black (*b*), jaunty (*j*), rickets (*rk*), pupal (*pu*), elbow (*el*), no-ocelli (*noc*), outspread (*osp*), Alcohol dehydrogenase (*Adh*) and reduced (rd). Justifications for map order not evident from the summary data shown here will be found in WOODRUFF and ASHBURNER (1979b) or will be published elsewhere. Full data are available from the senior author on request.

Adh; in fact, it is probably the immediate neighbor of *Adh* on its distal side. The phenotype of *osp* is very similar to the phenotype noted by AARON and all *Df(2L)A* deficiencies include *osp*. The wing phenotypes of all *Df(2L)A* deficiencies when heterozygous with an *osp* allele are identical to those of viable heterozygotes between two different deficiencies.

Closer study of viable combinations of different *Df(2L)A* deficiencies showed that some, but not all, expressed another phenotype: the flies lacked their ocelli and associated chaetae. This phenotype had previously been noted by WOODRUFF and ASHBURNER (1979a) in heterozygotes between, for example, *Df(2L)Sco*$^{R+7}$ and *Df(2L)fn3*. Viable combinations of *Df(2L)A* deficiencies and *Df(2L)Sco*$^{R+7}$ or *Df(2L)fn3* also lack their ocelli and associated chaetae. Table 2 shows the result of crossing *Df(2L)A* deficiencies *inter se*. Broadly speaking, the *Df(2L)A* deficiencies fall into three groups: those that include lethal loci both to the left and to the right of *Adh* (e.g., *Df(2L)A376*), those that include lethals only to the left of *Adh* (e.g., *Df(2L)A47*, a "left-hand" deletion) and those that include lethals only to the right of *Adh* (e.g., *Df(2L)A48*, *Df(2L)A267*, "right hand" deletions). All combinations of "left-hand" and "right-hand" deletion are viable and all are outspread and ADH$^-$. Some, but not all, also show the ocellar

TABLE 2

Viabilities of trans combinations of deletions

a) Left-hand deletions × right-hand deletions

	A48	%	*A267*	%	*A446*	%
A47	48/369	13.0	43/407	10.6*	0/612	0
A63	217/1266	17.1	154/716	21.5*	107/404	26.5
A178	187/942	19.9	241/1128	21.4*	54/1698	3.2*
A217	75/359	20.9	33/250	13.2*	0/107	0
A245	136/724	18.8	29/609	4.8*	0/199	0
A260	79/770	10.3	64/309	20.7*	0/309	0
A266	185/1262	14.7	92/775	11.9*	0/151	0
A379	220/1235	17.8	101/488	20.7*	17/494	3.4*
A445	87/679	12.8	116/509	22.8*	9/527	1.7*

b) Right-hand deletions × left-hand deletion

	A47	%
A48	48/369	13.0
A63	295/1150	25.7
A72	31/208	14.9
A178	214/1584	13.5*
A220	12/262	4.6
A264	13/324	4.0
A267	43/407	10.6*
A379	52/864	6.0*
A445	190/719	26.4*
A446	0/612	0

All viable combinations are osp^-. Those marked * are also noc^-. See text for explanations of left-hand and right-hand deletions.

phenotype. All combinations of left-hand deletions with *Df(2L)A48* have normal ocelli, whereas all combinations of these deletions with *Df(2L)A267* lack their ocelli. The "ocellar locus" therefore lies between the left-hand breakpoints of *Df(2L)A48* and *Df(2L)A267* and is included in all left-hand deletions. *Df(2L)A446* has rather a similar extent to *Df(2L)A48* except that it is lethal with the left-hand deletions, e.g., *Df(2L)A47*. This is caused by the fact that the left-hand limit of *Df(2L)A446* is distal to the lethal complementation group *l(2)br29* (defined by Sco^{R+1}).

It is evident that, because some of the *nLA* mutations (e.g., *nLA379*) include the ocellar locus but are viable with a left-hand deletion such as *Df(2L)A47* and with $l(2)br29^{ScoR+1}$, the ocellar locus lies between *l(2)br29* and *osp*, and that no vital loci are included in the *noc, osp, Adh* interval.

Using deletions that include the "ocellar locus," we have induced three mutations with EMS and ten with gamma rays that express the ocellar phenotype. We conclude this phenotype to result from mutation, or loss, of a locus we call no-ocelli (*noc*). All of the induced *noc* alleles map, by deletion mapping, to the region defined by the left-hand breakpoints of *Df(2L)A48* and *Df(2L)A267*. In addition, three are aberrations: the EMS-induced $In(2L)noc^2$, the gamma ray-induced ($In(2L)noc^4$) and the complex $T(Y;2)noc^7$, with breakpoints in 35B1.2, a

location consistent with the genetic data. There are several indications of genetic complexity in the *l(2)br29-noc* region. For example, the Sco^{R+1} allele of *l(2)br29* is lethal with some, but not all, *noc* alleles, despite the facts that (a) these *noc* alleles are either homozygous or hemizygous viable and (b) that the *l(2)br29* and *noc* loci are clearly separated by the *Df(2L)A267*, *nLA379* and *nLA445* breakpoints (see ASHBURNER, TSUBOTA and WOODRUFF 1982; M. ASHBURNER *et al.*, unpublished results and Table 5). The interaction between these loci is relevent to the interpretation of the nature of the left-hand breakpoints of *Df(2L)A446* and of three of the nLA mutations (*nLA178*, *nLA379* and *nLA445*). This will be discussed below.

All of the *Df(2L)A* deficiencies were induced on a chromosome mutant for black, therefore none can easily be tested for the inclusion of the *b* locus. However, only *Df(2L)A47* is lethal with *Df(2L)b80cl*, a small b^- deletion (*Df(2L)34D3;34D8—E1-2*) and none is lethal with the b^- deletion associated with the complex translocation *T(2;3)dp*. The *T(2;3)dp* deletion includes, as well as *b*, five lethal complementation groups near *b*. The lethality of *Df(2L)A47* and *Df(2L)b80c1* results from both of these being deleted for *l(2)br31*, a lethal complementation locus defined by two alleles recovered by J. O'DONNELL (unpublished).

Data relevant to the mapping of the distal breakpoints of those *Df(2L)A* deficiencies broken in the left-hand part of *Df(2L)64j* are shown in Table 3. *Df(2L)A376* includes rickets (*rk*) but not jaunty (*j*). *Df(2L)A376/Df(2L)*b^{75} heterozygotes are lethal as a result of *l(2)br30*, a lethal (defined by one EMS-induced allele, responsible for the "exceptional" lethality of *Df(2L)*b^{75}*/Df(2L)W* (WOODRUFF and ASHBURNER 1979a); our cytology was wrong) mapping between *rk* and *l(2)br1*. *Df(2L)*b^L, a deletion for 34D3;34E3-5 isolated by BRUCE BAKER, is similar to *Df(2L)*b^{75} except that it is *l(2)br30*$^+$. *Df(2L)*b^L*/Df(2L)A376* heterozygotes are viable but have a typical mutant rickets phenotype.

The nLA series: Several of the nLAs are homozygous viable or, if not, their lethality is caused by mutations of loci elsewhere on chromosome 2 (AARON 1979 and Table 4). Of the 14 *nLAs* studied, 10 show no phenotype (other than being black and ADH$^-$) when heterozygous with *Df(2L)64j* (Table 4). The four exceptions are *nLA63*, *nLA178*, *nLA379* and *nLA445*. Three of these are viable with *Df(2L)64j*, but the heterozygotes are both outspread and no-ocelli. (Note that their viability with *Df(2L)64j* is low, caused in part by the relative inviability of any genotype that expresses a strong *osp* phenotype, the flies have a great propensity for getting themselves stuck in the medium.) The fourth *nLA*, *nLA63*, is almost lethal with *Df(2L)64j*. This is caused by factors outside this genetic region, as can be shown by crossing *nLA63* to other deletions that include all of the lethals known to be in *Df(2L)64j*. Heterozygotes of *nLA63* with other large deletions (e.g., *Df(2L)75c*) are viable, and are *osp* but *noc*$^+$.

We conclude that 4 of the 14 *nLAs* are deletions. Three of these deletions include both *osp* and *noc* and the fourth (*nLA63*) only *osp* (in addition, of course, to *Adh*). These conclusions were confirmed by crossing these four mutations to point alleles of *noc* and *osp*. For the sake of consistency, we shall now call these mutations *Df(2L)A63*, *etc.*

TABLE 3

Mapping distal limits of Df(2L)A's *in the neighborhood of black*

	br32	b	br16	br17	br24	br31	j	rk	br30	br1	br15	T:dp	b80c1	b^{L}	b^{75}
A47	+	−	+	+	+	*0/255*	j	rk	1/217	*0/1160*	*0/189*	+	*0/641*	*0/369*	*0/266*
A376	+	−	+	+	+	+	+	rk	*0/228*	*0/591*	*0/357*	+	+	*52/392 rk*	*0/350*
A263	+	−	+	+	+	+	+	+	*0/155*	*0/365*	*0/602*	+	+	+	*0/599*
A215	−	−	+	+	+	−	+	+	−	*0/107*	*0/382*	−	−	+	+
A377	−	−	−	−	−	−	+	+	+	*0/201*	*0/146*	+	−	+	+
A246	−	−	−	−	−	−	+	+	+	*0/273*	*0/104*	+	−	+	+
A217	−	−	−	−	−	−	+	+	−	*0/107*	*0/1010*	+	+	+	+
A400	−	−	−	−	−	+	+	+	−	+	+	+	−	+	+
T(2;3)dp	*0/315*	b	*0/1014*	*0/257*	*899/3280*	+	+	+	+	+	+				
b80c1	*0/156*	b	*0/212*	*0/154*	*3/1318*	*22/474*	+	+	+	+	+				
b^{L}	*0/398*	b	*0/992*	*0/519*	*16/2123*	*23/685*	j	rk	+	+	+				
b^{75}	*0/411*	b	*0/1904*	*0/869*	*74/3852*	*20/693*	j	rk	*0/539*	+	+				

T(2;3)dp, *Df(2L)b80c1*, *Df(2L)*b^{L} and *Df(2L)*b^{75} are four b^{-} deletions with distal limits indicated by the data in this table (lower section). *l(2)br32* to *l(2)br15* is the 34D6 to 34F3 interval. Note that a homozygous rk^{-} deletion is viable (*Df(2L)A376 / Df(2L)*b^{L}). − indicates no data or test not easily possible. Most br24 alleles are leaky, the escapers having a small rough eye; *br24 / T(2;3)dp* is viable but shows this eye phenotype. *br31* alleles are also leaky, escapers having a thin bristle phenotype.

TABLE 4

Viability of nondeficient or small deficiencies with Df(2L)64j L^2

	N	%	Phenotype[a]
nLA2	129/340	37.9	+
nLA73	128/344	37.2	+
nLA74	136/458	29.7	+
nLA80	69/242	28.5	+
nLA248[b]	291/599	48.6	+
nLA249[b]	145/334	43.4	+
nLA252[b]	177/328	54.0	+
nLA319	136/401	33.9	+
nLA378	124/387	32.0	+
nLA405	125/444	28.2	+
b cn bw[b]	104/218	47.7	+
A63	2/326	0.6[c]	osp
A178	12/672	1.8	noc, osp
A379	24/365	6.6	noc, osp
A445	18/262	6.9	noc, osp

[a] All except 405 are *b*, and all are ADH^-.

[b] As homozygotes.

[c] The low viability of *A63/Df(2L)64j* is caused by unrelated factors: *A63* is not semilethal with other long deletions of region 34 – 35, eg., *A63/Df(2L)75c* have a relative viability of 25.6% ($n = 539$) and *A63/Df(2L)*b^{75} a relative viability of 42.2% ($n = 237$).

The dominant mutation Scutoid (*Sco*) was mapped, by recombination, to the right of *Adh* by O'DONNELL *et al.* (1977). Genetically it is a complex reciprocal transposition with the new gene order, el^+ *l(2)br22*$^+$ *l(2)br29*$^+$/(*rd*$^+$ *l(2)br34*$^+$ *l(2)br35*$^+$)/*l(2)br3*$^+$ . . . *l(2)br33*$^+$/Adh^F osp^+ noc^+/*l(2)br28*$^+$ *l(2)br36*$^+$ (ASHBURNER, TSUBOTA and WOODRUFF 1982). The Scutoid phenotype (a loss of macrochaetae from the head and thorax) may be a result of a "position effect" at the *noc/l(2)br28* junction and the phenotype is very sensitive to deletion or mutation in the region between (and including) elbow and no-ocelli. Depending upon just how much of this four-gene interval is deleted, the *Sco* phenotype is enhanced to a greater or lesser degree. When *Sco* is heterozygous with a wild-type chromosome, the flies (*Sco/+*) have between 24 and 29 (instead of the wild-type number of 40) bristles on their head and dorsal thorax. When heterozygous for a deletion for the entire region between, for example, pupal (*pu*) and *Adh* (e.g., *Df(2L)A47*), *Df/Sco* flies have only about 9 bristles. A smaller deletion, for example, *Df(2L)A245*, which is pu^+ el^+ *l(2)br22*$^-$ *l(2)br29*$^-$ noc^- osp^- Adh^-, enhances to a lesser extent, *Sco/Df(2L)A245* have 13 bristles per fly. *Df(2L)A267*, which is *l(2)br29*$^+$ noc^-, still enhances but to a lesser degree than *Df(2L)A245*, *Sco/Df(2L)A267* have 17 bristles per fly. A noc^+ deletion, for example, *Df(2l)A48* or *Df(2L)A72*, does not enhance *Sco*, or does so only very weakly.

These data (which are confirmed by crosses to deficiencies unrelated to the *A* series; ASHBURNER *et al.* 1982) are sufficient to indicate that the phenotype of *Sco* heterozygotes is a sensitive indicator of deletion of material in the region

immediately distal to *Adh*. All of the *Df(2L)A* and *nLA* series have been crossed to *Sco* and the phenotype of the heterozygotes scored by counting their bristles. Only those that had already been shown to be deletions by other criteria enhanced the *Sco* phenotype (Table 5).

Also shown in Table 5 are the viabilities of heterozygotes between *Df(2L)A* deficiencies and the X-ray-induced revertant of *Sco*, Sco^{R+1}, whose recessive lethal phenotype defines *l(2)br29*. Those deletions that enhance the phenotype of *Sco* by as much as an extra 15–20 bristles per fly (*i.e.*, 8–10 bristles per fly in the *Df/Sco* heterozygotes) are all lethal with Sco^{R+1}. Those that enhance the phenotype only by 10 bristles (or less) per fly are viable with Sco^{R+1}. Another X-ray-induced revertant of *Sco*, Sco^{R+27}, is also a *l(2)br29* allele, yet one that is somewhat "leaky" with *l(2)br29*$^-$ deletions and one that partially complements Sco^{R+1} with respect to viability (195 Sco^{R+1}/Sco^{R+27} heterozygotes in 2234 progeny of $Sco^{R+1}/CyO \times Sco^{R+27}$, *b pr/CyO*). With one exception, all *Df(2L)A* deficiencies that are lethal with Sco^{R+1} are also lethal, or very nearly so, with Sco^{R+27}. The exception is *Df(2L)A178*, $Df(2L)A178/Sco^{R+27}$ heterozygotes have 24.8% relative viability, $Df(2L)A178/Sco^{R+1}$ heterozygotes only 1.6% relative viability (Table 5).

The lethality of *Df(2L)A178* and Sco^{R+1} is paradoxical because *Df(2L)A178* homozygotes are far more viable (12.7%, see Table 7) than are $Df(2L)A178/Sco^{R+1}$ heterozygotes, and *Df(2L)A178* is reasonably viable with other *l(2)br29*$^-$ deletions (Table 6). Similar interactions are shown between *Df(2L)A379* and *Df(2L)A445* with *Df(2L)A446*. Both of these small deficiencies are viable with both Sco^{R+1} and Sco^{R+27} and are reasonably viable as homozygotes (Table 7) or when heterozygous with other *l(2)br29*$^-$ deletions, yet only a small percentage of their heterozygotes with *Df(2L)A446* survive (Table 2). Moreover, we have observed that when heterozygous with "point" *noc* alleles, *Df(2L)A446* flies show a more extreme ocellar phenotype than when the *noc* alleles are heterozygous with other *noc*$^-$ deletions, such as *Df(2L)A47*.

We interpret these data as indicating that the interactions between both the *A178* and *A446* deficiencies and Sco^{R+1} not only result from the fact that these mutations are deletions, but also from some particular properties of their left-hand break points. The fact that *Df(2L)A178* is not lethal with Sco^{R+27} suggests that the *l(2)br29* locus is complex and that the *A178* break point is not quite so far to the left as that of *Df(2L)A446*. We note that with respect to its enhancement of *Sco*, *Df(2L)A178* (but neither *Df(2)A379* nor *A445*) behaves as a *l(2)br29*$^-$ deficiency (Table 5).

Viability and fertility of homozygous deletion genotypes: Table 7 shows the relative viabilities of the four "small" *Df(2L)A* deficiencies *i.e.*, *A63*, *A178*, *A379* and *A445*. With the exception of *Df(2L)A63*, all are reasonably viable; the fact that their viability is less than that expected can be accounted for by their strong outspread wing phenotype (see above). The inviability of *Df(2L)A63* is caused by factors outside this chromosome region (see above); thus a homozygous deletion of three adjacent autosomal loci is viable. But are they fertile? Homozygous male and female *Df(2L)A178*, *A379* and *A445* were all outcrossed to Canton-S, as five-pair cultures, and all proved to be both male and female

TABLE 5

Interaction of A and nLA series with Sco and two Sco revertants

	Sco	%	$\bar{x}^a$	Sco R+1	%	Sco R+27	%
Df(2L) el⁻br22⁻br29⁻noc⁻osp⁻							
A47	419/3072	13.6	8.80 ± 0.25	0/139	0	5/235	2.1
A215	16/354	4.5	9.00 ± 0.26	—		—	
A217	58/915	6.3	10.27 ± 0.38	0/198	0	3/112	2.7
A246	1/454	0.2	—	0/534	0	0/306	0
A263	80/1153	6.9	9.45 ± 0.28	0/295	0	3/207	1.4
A376	21/627	3.3	11.25 ± 0.41	0/162	0	0/159	0
A377	1/391	0.3	8 —	0/444	0	0/261	0
A400	18/310	5.8	10.21 ± 0.38	0/198	0	0/347	0
Df(2L) br22⁻br29⁻noc⁻osp⁻							
A245	117/982	11.9	13.20 ± 0.43	0/874	0	4/215	1.9
A260	195/1316	14.8	11.87 ± 0.37	0/319	0	42/495	8.5
A266	158/913	17.3	12.25 ± 0.32	0/253	0	1/204	0.5
Df(2L) br29⁻noc⁻osp⁻							
A446	138/1445	9.6	12.20 ± 0.27	9/1863	0.5	2/399	0.5
A178	672/3104	21.6	12.55 ± 0.41	43/2764	1.6	112/451	24.8[b]
Df(2L) noc⁻osp⁻							
A267	153/600	25.5	17.30 ± 0.46	159/784	20.3	280/1139	24.6
A379	222/940	23.6	17.40 ± 0.42	89/368	24.2	82/322	25.8
A445	361/1242	29.1	17.60 ± 0.63	91/343	26.5	59/239	24.7
Df(2L) osp⁻							
A48	214/986	21.0	22.40 ± 0.33	370/2949	12.5	30/784	3.8[b]
A63	102/352	29.0	24.45 ± 0.37	88/311	28.3	116/423	27.4
A72	250/723	34.6	24.70 ± 0.48	355/1052	33.7	265/765	34.6
A220	166/516	32.2	23.05 ± 0.41	140/620	22.6	85/309	27.5
A264	241/836	28.8	22.05 ± 0.57	233/922	25.3	26/115	22.6
osp⁺							
nLA2	79/223	35.4	25.15 ± 0.40	—		—	
nLA73	130/418	31.1	26.15 ± 0.37	—		—	
nLA74	180/531	33.9	25.35 ± 0.42	—		—	
nLA80	122/422	28.9	25.35 ± 0.36	—		—	
nLA248[c]	317/704	45.0	25.80 ± 0.63	—		—	
nLA249[c]	232/482	48.1	26.45 ± 0.44	—		—	
nLA252[c]	177/396	44.7	24.85 ± 0.46	—		—	
nLA319	158/410	38.5	25.75 ± 0.37	—		—	
nLA378	110/371	29.6	26.65 ± 0.65	—		—	
nLA405	227/779	29.1	27.00 ± 0.50	195/591	33.0	115/464	24.8
b cn bw[c]	233/446	52.2	24.95 ± 0.36	171/371	46.1	—	

The deletions are grouped according to the position of their distal break points relative to the *el* to *osp* interval.

[a] Mean ± S.E. bristle number of 10 males and 10 females unless fewer recovered.

[b] See text.

[c] As homozygotes, others balanced over CyO, Adh^{nB}.

TABLE 6

Viability of Df(2L)A178 *and* l(2)br29 *alleles with* l(2)br29$^-$ *deletions*

	A178	%	*Sco*$^{R+1}$	%	*Sco*$^{R+27}$	%
Sco^{R+1}	—	—	—	—	195/2234	8.7
A178	62/487	12.7	43/2764	1.6	112/451	24.8
Df(2L)fn2	36/435	8.3	0/528	0	36/796	4.5
Df(2L)fn3	142/685	20.7	0/1008	0	65/662	9.8
Df(2L)fn7	76/326	23.3	0/1936	0	17/366	4.6
Df(2L)AR-R1	56/423	13.2	0/313	0	17/431	3.9
Df(2L)C75RL	31/252	12.3	0/530	0	4/387	1.0
Df(2L)W	60/396	15.2	0/212	0	17/302	5.6

Data from *Df(2L)A178/CyO* or *l(2)br29/CyO* × *Df/(2L)CyO*.

TABLE 7

Homozygous and heterozygous viability of small deletions

	A63	*A178*	*A379*	*A445*
A63	0.8 (528)			
A178	21.1 (1140)	12.7 (487)		
A379	23.4 (1182)	21.6 (1693)	13.9 (683)	
A445	19.3 (715)	23.3 (1495)	19.6 (414)	13.7 (563)

All flies are *osp*$^-$*Adh*$^-$ and also *noc*$^-$, unless they carry *Df(2L)A63*. Data from *Df(2L)/CyO* × *Df(2L)/CyO*. % viability (*n*).

fertile, although by no means as fertile as, for example, *nLA405* homozygotes. Nevertheless, there can be no loci within the *noc* to *Adh* interval whose integrity is required for either male or female fertility. This conclusion was confirmed by testing the fertility of these four small deletions when heterozygous with either *Df(2L)A47* or *Df(2L)A48*.

ADH phenotypes: All of the nLAs were crossed to *Df(2L)64j* and heterozygous progeny assayed for ADH activity spectrophotometrically. All were ADH null, showing no sign of any enzyme activity.

DISCUSSION

Twenty-eight X-ray-induced and two spontaneous mutations of *Adh*, all selected on the basis of resistance to the toxic effects of 1-penten-3-ol have been included in this study. Of the 28 X-ray-induced alleles, 20 are deletions of *Adh* and of at least one neighboring locus. One of the two spontaneous mutations is also a deletion. This contrasts with 13 EMS-induced alleles of *Adh* that we have studied (M. BODMER, unpublished). None of these are deletions, at least none are deletions extending into known loci neighboring *Adh* and 11/13 make translatable ADH mRNA (R. KARP, M. BODMER and H. PELHAM, unpublished).

One possible bias in these data should be discussed. It may be that the chemical screen used to recover *Adh* mutations selected against mutant alleles that were "leaky," *i.e.*, mutations resulting in a greatly reduced but not totally absent ADH activity. Then the screen would have been biased in favor of

mutations that completely abolished all ADH activity. This bias has been previously reported (SOFER and HATKOFF 1972). It is relevant that of the seven EMS-induced alleles we or SCHWARTZ and SOFER (1976) have studied that were *not* selected by pentenol screens, but by a histochemical test, (*i.e.*, Adh^{n1} to Adh^{n5}, Adh^{nC1} and Adh^{nC2}), three [*i.e.*, Adh^{n5} (VIGUE and SOFER 1974), Adh^{nC1}, and Adh^{nC2} (M. ASHBURNER, unpublished results)] are "leaky" alleles, showing residual ADH activity, and two (Adh^{n5} and Adh^{nC1}) are markedly temperature sensitive for their ADH activity (VIGUE and SOFER 1974; C. DETWILER, unpublished). Of nine EMS-induced alleles selected by a pentenol screen (GERACE and SOFER 1972; SOFER and HATKOFF 1972) none were leaky (SCHWARTZ and SOFER 1976).

The interesting question, however, is what proportion of intragenic mutations of *Adh* generated with X rays are single-site, and what proportion are deletions not extensive enough to enter neighboring loci. GELBART *et al.* (1974) are firmly of the opinion, largely based upon the results of the reversion properties of *ad-3B* mutations of *Neurospora crassa* (MALLING and DE SERRES 1973), that "in contrast to prevailing scientific folklore ... single-site mutations comprise the bulk of intragenic events produced by X-irradiation". Of the ten X-ray-induced mutations that we can class as being "intragenic", two make no translatable ADH mRNA (R. KARP, M. BODMER and H. PELHAM, unpublished). Because we have *prima facie* grounds for believing that X rays may generate nonsense mutations [*i.e.*, *nLA2* and *nLA74*, whose ADH polypeptide is smaller than normal (M. BODMER, R. KARP and H. PELHAM, unpublished)], these messenger RNA-negative mutations may, of course, be nonsense mutations so near the N-terminus of the polypeptide that their translation products are undetectable. Alternatively, *nLA2* and *nLA74* (and the two messenger RNA-negative alleles) may be internal deletions within *Adh*. Only further molecular studies can distinguish these possibilities and further speculation is not warranted. We note, however, that at the rudimentary locus the spectrum of mutations induced by EMS and X rays also differs. RAWLS and PORTER (1979) estimate that 86% of EMS-induced, but only 53% of X-ray-induced, r mutations are complementing alleles. It is quite clear, from these data and from our own studies of EMS- and X-ray-induced *Adh* alleles, that the types of mutation induced by these agents differ, on average. This does not mean that both agents may not, on occasion, produce the "same" class of mutational lesion.

O'DONNELL *et al.* (1977) and ourselves (WOODRUFF and ASHBURNER 1979b, this study) have now mapped the extents of 21 X-ray-induced deletions selected on *Adh* (with pentenol) and 12 formaldehyde-induced deletions selected on *Adh* (with 1-pentyn-3-ol, O'DONNELL *et al.* 1975). It is interesting to compare the frequencies of recovery of these deletions with regard to the idea that formaldehyde is a "good" mutagen to use in Drosophila if small deletions are required. In the formaldehyde experiments 67% (12/18) of the Adh^- mutations were deletions for at least one locus neighboring *Adh*. In the X-ray study, 71% (20/28) Adh^- mutations were deletions by the same criterion. In view of the facts that from the practical point of view formaldehyde is far more difficult a mutagen to use than X rays and the frequency of induced Adh^- mutations is far higher

after 3000 r of X rays than after larval feeding of formaldehyde [(19/86,900 (*i.e.*, 1:4573) fertile Adh^- after 3000 r X rays (AARON 1979), 7/350,000 and 14/790,000 (i.e., approximately 1:50,000) fertile Adh^- after larval feeding formaldehyde (O'DONNELL *et al.* 1975, 1977)], we cannot recommend the use of formaldehyde as a mutagen in Drosophila, if the objective is only to recover deletions. (We would be rather surprised if the tenfold difference in recovery of X ray and formaldehyde included mutations of *Adh* seen here is wholly caused by differences in selection technique.)

With respect to the distribution of deletion breakpoints on the genetic map of the 34D-35D region, both the X ray and the formaldehyde deletions show evidence of break point clustering. That the distribution of deletion breakpoints is nonrandom with respect to the genetic map was discussed by WOODRUFF and ASHBURNER (1979a). The data that have been collected since the study only confirm the conclusions then drawn. Moreover, we can now see that the clustering is similar for both sets of deletions; there is no clear evidence that one or the other mutagen "prefers" a particular region for breakage (Fig. 1). With respect to deletion size we note that all of the smallest deletions we have studied were X ray induced; indeed there are five X-ray-induced deletions smaller than *Df(2L)fn2* and *Df(2L)fn3*, the smallest formaldehyde-induced deletions. The mean size of the X-ray-induced deletions is 13.05 bands (12.76 complementation groups). For the formaldehyde-induced deletions the mean size is 21.09 bands (18.42 complementation groups). In view of the relatively small numbers of X-ray- and formaldehyde-induced deletions studied, we do not attribute any special significance to these differences.

There is, however, one difference between the two sets of mutations. None of the formaldehyde-induced *Adh* deletions were associated with any other chromosomal mutation [we cannot confirm the suggestion (O'DONNELL *et al.* 1977) that *Df(2L)fn36* has an aberration in addition to its deletion]. Two of the X-ray-induced alleles, *Df(2L)A379* and *Df(2L)A446*, were associated with complex chromosome breaks in the region of *Adh* itself, indeed the *A379* deletion is associated with an inversion break point. In addition, three other mutations were associated with one or more quite independent aberrations.

We have, to a certain extent, discussed the complications in the genetic analysis of the region immediately distal to *Adh* in the RESULTS section of this paper. The organization of the elbow to no-ocelli region is bizarre and we will not discuss the matter further here (but will do so in another publication) except to point out that two of the deletions whose left-hand break points fall into this region, (*A178* and *A446*), exhibit properties that lead us to conclude that a very specific phenotype is associated with their break points *per se*. This is, of course, not unexpected on general grounds, but it does warn against a too simplistic interpretation of deletion mapping data.

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