EVIDENCE FOR A SET OF CLOSELY LINKED AUTOSOMAL GENES THAT INTERACT WITH SEX-CHROMOSOME HETEROCHROMATIN IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

It is proposed that there exists a special region in the euchromatin of the left arm of chromosome *2* (contained within sections 31–32 of the standard salivary gland chromosome map) that is defined by a set of genes, each one of which interacts with a specific sex-chromosome heterochromatic segment. The evidence for the existence of this region is, first, the exhibition, mapping, and analysis of five different maternal-effect, embryonic semi-lethals located in region 31–32. Secondly, in each case the consequence of the maternal effect is markedly influenced by the amount of *X*- or *Y*-chromosome heterochromatin carried by the progeny of mutant mothers. The nature of this interaction and possible reasons for the existence of the cluster of autosomal genes are discussed.

EVIDENCE has been presented previously that two second-chromosome recessive hypomorphic maternal-effect mutants, daughterless (*da*; BELL 1954) and abnormal oocyte (*abo*; SANDLER *et al.* 1968), are closely linked and that each interacts with, and possibly regulates, a specific sex-chromosome heterochromatic segment (SANDLER 1970, 1972, 1975; MANGE and SANDLER 1973; MASON 1973; PARRY and SANDLER 1974). In this report, data are presented that suggest that the linkage of *da* and *abo* is not happenstance, but rather implies and locates a euchromatic region on chromosome *2* that contains a cluster of autosomal genes that interact with sex-chromosome heterochromatic segments.

RESULTS

To recover new *da–abo*-like mutants in the *da–abo* region, advantage was taken of the observations that *da*, heterozygous with a deficiency for *da*, is lethal (MANGE and SANDLER 1973) and that *abo*, heterozygous with a deficiency for *abo*, has a substantially reduced viability (SANDLER 1975). Advantage was also taken of the existence of a deficiency, *Df(2L)J-der-39*, that includes both the *da* and *abo* loci (SANDLER 1975). All mutants and all abnormal chromosomes utilized in the experiments reported here, except for *abo* and the deficiencies in the *da–abo* region, are described in LINDSLEY and GRELL (1968).

Accordingly, Canton-S second chromosomes were treated with ethyl methanesulfonate, put through at least one generation of outcrossing to resolve mosaics,

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and then females carrying the treated second chromosome heterozygous with *In(2LR)Cy* were mated to *Df(2L)J-der-39/Sco* males. In this way some 455 treated second chromosomes were examined for cases that exhibited a markedly reduced survival when heterozygous with the deficiency. Five second chromosomes were ultimately recovered that survived poorly with the deficiency in the original tests and in larger retests; the summed results of the tests of these five chromosomes, along with a control and with identical tests of *da* and *abo*, are given in Table 1. It can be seen that the *M* class (the treated chromosome *2* heterozygous with the deficiency) is at least as viable as the *Cy M* class [*In(2LR)Cy/Df(2L)J-der-39*] in wild-type controls, but is markedly less viable in the case of *da*, *abo*, and the five new mutants recovered in this experiment—*da-abo*-like (*dal*), wavoid-like (*wdl*), hold-up (*hup*), *mfs48*, and *l(2)54*.

The five new mutants were made heterozygous with chromosomes from a standard inbred wild-type strain (Canton-S) in females for one or two generations to allow free recombination, and a mutant-bearing chromosome *2* was reisolated from each line and kept heterozygous with a balancer second chromosome, *In(2LR)Cy*. From crosses among these stocks, and among *da/In(2LR)Cy* and *abo/In(2LR)Cy* stocks, attempts were made to recover the seven mutants as homozygous females, and also to recover all possible heterozygotes as females. These females were examined for visible phenotype and crossed to males carrying normal second chromosomes, an attached-*XY* chromosome, and no other sex chromosome. Attached-*XY* males were used because in the case of *abo* such males result in the maternal effect producing the most aberrant sex ratio. The results of these crosses are shown in Table 2.

It is seen that *l(2)54* is lethal not only over a deficiency, but also when homozygous. Attempts to separate the two lethalities failed; *l(2)54* is, therefore, probably simply a lethal located within the limits of *Df(2L)J-der-39*.

The mutant *mfs48* has a short-bristle phenotype and proved to be sterile both as homozygous females and as homozygous males. Extensive tests to separate the

TABLE 1

Results of crosses of females heterozygous for In(2LR)Cy *and the indicated second chromosome by* Df(2L)J-der-39*/Sco *males*

Second chromosome	Phenotype of progeny											
		Cy Sco			*Sco*			*Cy M*			*M*	
	♀♀	♂♂	Σ	♀♀	♂♂	Σ	♀♀	♂♂	Σ	♀♀	♂♂	Σ
+	975	1015	1990	938	1010	1948	459	570	1029	552	697	1249
da	793	806	1599	771	731	1502	467	465	932	4	6	10
abo	363	377	740	326	378	704	228	265	493	147	232	379
hup	4039	3970	8009	3491	3321	6812	2497	2311	4808	1	1	2
wdl	3441	3241	6682	2849	2787	5636	1780	1704	3484	200	41	241
dal	972	948	1920	838	860	1698	342	363	705	151	105	256
mfs48	2310	2139	4449	1733	1847	3580	1362	1392	2754	0	4	4
l(2)54	1213	1212	2425	1067	1175	2242	718	759	1477	0	2	2

* The deficiency includes a female-sterile Minute locus, *M(2)fs*.

TABLE 2

Tests of allelism: crosses of females carrying the indicated second chromosomes by YSX·YL, In(1)EN, y B/O *males**

Genotype of females	Reg. ♀♀	Reg. ♂♂	Exc. ♀♀	Exc. ♂♂	Percent reg. ♀♀	No. progeny per mother
+/+	3066	3681	2	0	45	224.9
hup/hup	258	478	2	1	35	1.7
wdl/wdl	1103	694	1	1	61	7.7
mfs48/mfs48	—	—	—	—	—	—
l(2)54/l(2)54	—	—	—	—	—	—
dal/dal	1567	1170	2	2	57	38.5
abo/abo	2261	233	4	2	91	83.1
da/da	0	3331	0	2	00	55.5
hup/wdl	2535	3046	0	2	45	253.7
hup/mfs48	6019	8560	5	5	41	243.0
hup/l(2)54	2890	4109	2	4	41	189.0
hup/dal	2990	4094	0	3	42	244.3
hup/abo	2807	3542	8	14	44	211.6
hup/da	2923	4054	0	9	42	232.6
wdl/mfs48	4976	6730	0	5	43	195.1
wdl/l(2)54	3171	3973	0	1	44	238.1
wdl/dal	3073	4246	0	4	42	209.1
wdl/abo	3006	3613	4	18	45	220.6
wdl/da	2836	3771	2	4	43	220.2
mfs48/l(2)54	2822	3672	2	1	43	216.5
mfs48/dal	3499	4890	1	1	42	233.0
mfs48/abo	2994	3733	8	5	45	224.2
mfs-48/da	3286	4732	1	4	41	267.3
l(2)54/dal	2156	2754	0	4	44	144.4
l(2)54/abo	1737	2281	0	0	43	211.5
l(2)54/da	1206	1898	0	0	39	238.8
dal/abo	2338	2917	0	1	44	262.7
dal/da	1591	2249	0	0	41	192.0
abo/da	3032	4569	0	4	40	253.4

* *mfs48* is a recessive female sterile; *l(2)54* is a recessive lethal.

bristle phenotype, the female sterility, and the male sterility from one another failed. Thus, *mfs48* appears to be a short-bristled, male-female-sterile mutation that is lethal over a deficiency for the locus. Owing to the lethality of *l(2)54* and sterility of *mfs48*, neither mutant could be tested further; it is, therefore, not known whether either of these genes forms part of the special *da-abo* region inferred from the other three mutants as described below.

Most important for the issue under consideration here, *hup*, *wdl*, and *dal*, like *da* and *abo*, survive as homozygotes, are female semi-sterile (as measured by the number of progeny per mother), and the progeny of homozygous females exhibit an abnormal sex ratio. Determinations of egg hatch show that the semisterility of females homozygous for the mutations is a consequence of the failure of eggs to hatch. In the case of *hup*, egg hatch was four percent that of heterozygous

(sister female) controls; it was eight percent in the case of *wdl*, and fifty percent for *dal*. With respect to morphology, *hup* exhibits an almost completely penetrant upheld-wing phenotype; *wdl* shows a wavy-wing morphology and an incomplete fifth longitudinal vein, and it may indeed be an allele of the now-lost mutant "wavoid" (*wd*) reported by KELLEN (1945), who mapped the mutant to position 40 on chromosome *2*; *dal*, like *da* and *abo*, is morphologically normal. We may also note here that the chromosomes carrying the mutants are all cytologically normal in salivary gland preparations.

Finally, the results of the crosses involving heterozygotes show that all seven mutations complement completely and, therefore, almost surely define seven different genes.

Analysis of the maternal-effect mutants: The three new mutants in the *da-abo* region that are homozygous viable and female semisterile—*hup*, *wdl*, and *dal*—are similar to *da* and *abo* in that all three are recessive, hypomorphic, maternal-effect mutants that result in zygotic mortality before egg hatch. The most striking property, however, of *da* and *abo* is that the zygotic mortality resulting from each of these mutants can be reduced by increasing the amount of *X*- or *Y*-chromosome heterochromatin carried by the zygote. This heterochromatic variation, furthermore, is achieved by varying the paternal heterochromatic contribution, implying that this heterochromatin is effective in rescuing a zygote after the egg has been completed. The first question to be asked about the new mutants, therefore, is whether they too interact with sex-chromosome heterochromatin in this way.

Accordingly, females carrying each of the three mutants, either homozygous or (sister females) heterozygous with *In(2LR)Cy*, were crossed to males carrying normal second chromosomes and (a) an attached-*XY* chromosome and no other sex chromosome, (b) an attached-*XY* chromosome and a free *Y* chromosome, or (c) normal sex chromosomes. This permits, by comparing the effect of each mutant on *XXY* ♀♀ vs. *XO* ♂♂, *XXY* ♀♀ vs. *XY* ♂♂, and *XX* ♀♀ vs. *XY* ♂♂, an assessment of the interaction between the maternal effect of the mutant and the *Y* chromosome in zygotes of either sex. The results of these crosses, and the indicated comparisons, are presented in Table 3.

To examine the effect of *X*-chromosome heterochromatin (*Xh*) on the phenotype of the mutants, females of the types just described were crossed to males carrying normal second chromosomes, a $B^{s}Y$ chromosome and an $In(1)sc^{4L}sc^{8R}$, *y cv v f X* chromosome. This *X* chromosome (symbolized X^{-}) is deficient for approximately the middle six- to seven-eighths of *Xh* including the *bb* locus, *NO*, and the pairing sites that assure the regular separation of the *X* and *Y* chromosomes. [For a review of the genetic properties of $In(1)sc^{4L}sc^{8R}$, see ZIMMERING (1976).] From these crosses, it is possible to examine the interaction between the maternal effect produced by the maternal-effect mutants and the presence of but one dose of *Xh* in female progeny, both with and without a *Y* chromosome. The results are given in Table 4.

The "♂-recovery" values (which are measures of the survival of males relative to females in the experimental crosses, corrected for the control sex ratio)

TABLE 3

Results of crosses of females carrying the indicated mutant, either homozygous (E) or heterozygous with In(2LR)Cy *(C), by males carrying normal second chromosomes and the indicated sex chromosomes*†

Maternal chromosome 2	Paternal sex chromosome		Progeny Reg. ♀♀	Reg. ♂♂	Exc. ♀♀	Exc. ♂♂	♂-rec.*
hup	*XY/O*	E	258	478	2	1	1.52
		C	1364	1659	1	4	
	XY/Y	E	206	235	0	1	1.19
		C	2884	2756	2	4	
	X/Y	E	511	376	1	4	0.73
		C	3238	3243	2	2	
wdl	*XY/O*	E	1103	694	1	1	0.46
		C	1266	1729	0	0	
	XY/Y	E	401	266	0	2	0.65
		C	3121	3170	0	2	
	X/Y	E	420	315	0	1	0.76
		C	3387	3322	4	4	
dal	*XY/O*	E	1567	1170	2	2	0.60
		C	2648	3269	3	5	
	XY/Y	E	1656	1102	2	2	0.71
		C	2816	2650	7	7	
	X/Y	E	3060	2308	0	5	0.78
		C	2671	2580	3	3	

† *XY* = *YSX·YL, In(1)EN, y B*; *X* = a normal *X* chromosome marked by *B*.
* "♂-recovery" is computed as [(♀♀ in Control) × (♂♂ in Experimental)] ÷ [(♀♀ in Experimental) × (♂♂ in Control)].

shown in Table 3 imply that for all three mutants the consequence of the maternal effect is influenced by whether or not male or female progeny carry a *Y* chromosome. In the case of *hup*, the presence of a *Y* chromosome increases the likelihood of *hup*-induced zygotic mortality; for *wdl* and *dal* this likelihood is decreased. Thus, from *hup* mothers relative to controls, *XO* males are recovered 1.3 (1.52/1.19) times as often as are *XY* males, or, using the corresponding data from Table 4, 1.4 times as often. For *wdl*, these numbers are 0.71 and 0.82, while for *dal* they are 0.85 and 0.61.

In the case of females, the data in Table 3 imply that *XX* female progeny of *hup* mothers, relative to controls, are recovered 1.6 (1.19/0.73) times as often as

TABLE 4

Results of crosses of females carrying the indicated second chromosome, either homozygous (E) or heterozygous with In(2LR)Cy *(C), by males carrying* In(1)sc^{4L}sc^{8R}, y cv v f/B^{s}Y

Phenotype of progeny	Chromosome type*		Chromosome 2 *hup*	*wdl*	*dal*
+ ♀♀	*XX*$^{-}$	C	1514	1730	2124
		E	336	303	1093
B^{s} ♂♂	*XY*	C	671	775	925
		E	106	87	472
B^{s} ♀♀	*XX*$^{-}$*Y*	C	175	237	263
		E	21	36	201
+ ♂♂	*XO*	C	2039	2301	3118
		E	450	213	969

* $X^{-} = In(1)sc^{4L}sc^{8R}$.

XXY females, while (from Table 4) *XX*$^{-}$ females are recovered 1.8 times as often as *XX*$^{-}$*Y* females. In the case of *wdl*, these recoveries are 0.86 and 1.2, while for *dal* they are 0.91 and 0.67. Thus, except for the relative recovery of *XX*$^{-}$ vs. *XX*$^{-}$*Y* females from homozygous *wdl* mothers, all the data indicate a consistent effect of the *Y* chromosome on the survival of the progeny of either sex of mutant mothers. For *wdl* and *dal*, the *Y* rescues zygotes from the *dal*-induced defect, while the *hup*-induced defect is enhanced by a *Y* chromosome. The one exceptional relationship involving *wdl* is not understood; it may be spurious (the numbers were quite small) or it may imply a complex interaction between *Xh* and *Y*.

To examine for a possible interaction between *Xh* and the maternal effect, we may compare the recovery, from mutant mothers relative to controls, of *XX*$^{-}$ females to *XY* males (from the data in Table 4) to the recovery, computed in the same way, of *XX* females to *XY* males (from the data in Table 3). For the case of *hup*, both *XX* and *XX*$^{-}$ females are recovered 1.4 times as often as *XY* males, implying that *Xh* does not interact with the *hup*-induced maternal effect. In the case of *wdl*, *XX* females are recovered less well, relative to *XY* males, (1.3 ×) than are *XX*$^{-}$ females (1.6 ×), implying that *Xh* increases the probability that a zygote will succumb to the *wdl*-induced egg abnormality. Finally, *XX* female progeny of *dal* mothers are recovered 1.3 times as often as are *XY* males, while *XX*$^{-}$ females are recovered as often as are *XY* males, implying that *Xh* rescues female zygotes from the *dal*-induced lethality.

In summary, then, *hup*, *wdl*, and *dal*, are like *da* and *abo* in being recessive, hypomorphic, maternal-effect mutants that interact with sex-chromosome heterochromatin in the zygote.

The progeny of homozygous *da* and *abo* males, in contrast to females, exhibit no interaction with sex-chromosome heterochromatin. To test the behavior of males carrying the new maternal-effect mutants, homozygous mutant males, and

brothers that were heterozygous for the mutant and *In(2LR)Cy*, were crossed to females carrying normal second chromosomes and one of three different sex-chromosome constitutions. (It should be noted that while homozygous *wdl* and *dal* males appear fully fertile, *hup* homozygotes are partially male sterile. It has not been determined whether this partial sterility is an effect of *hup* itself or of some other mutant on the *hup*-bearing chromosome 2.) The specific sex chromosomes carried by the parental females and the results of these crosses are recorded in Table 5. It can be seen that irrespective of the sex-chromosome constitution of the progeny, recovery is the same from mutant and nonmutant fathers. Thus the new maternal-effect mutants, like *da* and *abo*, exhibit no paternal effect.

Finally, *da* and *abo* exhibit one other similarity: in the case of each mutant, the zygotic mortality owing to the maternal effect is less when the eggs of mutant mothers develop at a temperature lower than the normal temperature of 25°. This has been shown for *abo* by SANDLER (1970) and for *da* by CLINE (1975, 1976). To examine this property for the three mutants under study here, a ♂-recovery value for each mutant was determined in crosses to attached-*XY/O* males, where the crosses were made and the progeny allowed to develop at 19°. These results are shown in Table 6; they may be compared to the results (previously presented in Table 3) of these same crosses at 25°. In each case, ♂-recovery is closer to 1.0 at the lower temperature, implying that the three new mutants, like *da* and *abo*, have a temperature-sensitive phenotype.

In summary, then, it has been shown that the three new mutants are like *da* and *abo* in six respects: (1) all are recessive hypomorphic mutations as evidenced by lethality or semilethality when heterozygous with a deficiency, survival in homozygous condition, and wild-type behavior when heterozygous with a normal allele; (2) none has any paternal effect; (3) all show sex-differential mortality as evidenced by unequal recovery of *XX* females and *XY* males from homozy-

TABLE 5

Results of crosses of males carrying normal, unmarked sex chromosomes and the indicated second chromosome either heterozygous with In(2LR)Cy *(Control) or homozygous (Experimental) by females carrying normal second chromosomes and the indicated sex chromosomes*

Constitution of parental female		♀♀	*hup* ♂♂	♂♂/total	♀♀	*wdl* ♂♂	♂♂/total	♀♀	*dal* ♂♂	♂♂/total
y/y	Cont.	1887	1811	0.49	1272	1152	0.48	3066	3127	0.50
	Exp.	2921	2732	0.48	427	462	0.52	3491	3728	0.52
C(1)DX,y f/Y*	Cont.	1279	1435	0.53	2045	2460	0.55	1844	2150	0.54
	Exp.	797	905	0.53	1352	1553	0.53	2048	2297	0.53
C(1)RM,y pn v/O	Cont.	1279	1435	0.53	1491	1552	0.51	1663	1790	0.52
	Exp.	939	932	0.50	814	801	0.50	2018	2287	0.53

* *C(1)DX,y f* is a *bb*-deficient reversed acrocentric compound-*X* chromosome homozygous for *y* and *f*.

TABLE 6

Results of crosses, carried out at 19°, of females carrying the indicated mutant, either homozygous (Exp.) or heterozygous with In(2LR)Cy *(Cont.), by males carrying normal second chromosomes,* YSX·YL, In(1)EN, y B, *and no other sex chromosome*

Progeny	*hup*		*wdl*		*dal*	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
Regular ♀ ♀	1307	1751	971	87	1315	1934
Regular ♂ ♂	1503	2251	1048	82	1773	1926
Exceptional ♀ ♀	4	4	1	0	2	2
Exceptional ♂ ♂	1	1	1	0	1	2
♂-recovery*		1.12		0.87		0.74
♂-recovery†		1.52		0.46		0.60

* "♂-recovery" is computed from these data as in Table 3.
† At 25°; computed from the data in Table 3.

gous mothers; (4) all produce a maternal effect resulting in zygotic mortality before egg hatch; (5) the consequence of the maternal effect, in each case, is affected by the *X*- or *Y*-chromosome heterochromatic content of zygotes developing from eggs produced by mutant mothers; and (6) the consequence of the maternal effect is, in all cases, less severe at 19° than it is at the normal rearing temperature of 25°.

Further analysis of dal: There are two additional comparisons that can be made between these mutants on the one hand and *da* and *abo* on the other. First, we may inquire whether the differential recoveries observed with different doses of *Xh* and *Y* in the progeny of mutant mothers are entirely the result of differential egg mortality as in the cases of *da* and *abo*. Secondly, the probability of surviving the maternally induced abnormality, for both *da* and *abo*, is increased by the presence of a wild-type allele of the maternal-effect mutant in the zygote; we may ask whether this also obtains for the new mutants. Unfortunately, *hup* and *wdl* homozygous females are too sterile to permit performing the required experiments; therefore, these two matters are examined only for the case of *dal*.

To examine the basis of the differential progeny recoveries from homozygous *dal* mothers, sister females, either homozygous or heterozygous for *dal*, were crossed to either attached-*XY/O* males or attached-XY/Y males, and both eggs and adults counted; the results are shown in Table 7. It can be seen that, as expected, ♂-recovery is markedly higher in crosses to males carrying a free *Y* chromosome (0.94) than in crosses to attached-XY/O males (0.48). The absolute (although relative to controls) survival by sex, computed by using the egg counts, shows that the difference in ♂-recovery is wholly the result of the reduced survival of *XO* males (0.21) compared with *XY* males (0.41); the *XXY* females exhibit the same survival (0.43–0.44) in both crosses.

To examine whether a *dal*$^+$ allele in the developing zygote increases the probability of that zygote surviving the *dal*-induced abnormality in the egg, the survival of *dal/dal*$^-$ zygotes relative to *dal*/+ zygotes from *dal* homozygotes was compared with the relative survival of these same zygotes from heterozygous

TABLE 7

The results of crosses of females carrying normal X *chromosomes and either homozygous for* dal *(Exp.) or heterozygous for* dal *and* In(2LR)Cy *(Cont.), by males carrying normal second chromosomes and the indicated sex chromosomes*†

	Parental male			
	XY/O		*XY/Y*	
	Cont.	Exp.	Cont.	Exp.
Number of eggs	300	300	300	400
Regular ♀ ♀	102	45	123	71
Regular ♂ ♂	136	29	112	61
Exceptional ♀ ♀	0	0	0	0
Exceptional ♂ ♂	0	0	0	1
♂-recovery*	0.48		0.94	
♀-survival	0.44		0.43	
♂-survival	0.21		0.41	

Eggs and adults were counted and absolute survival by sex computed.
* Computed as in Table 3.
† *XY = YSX·YL, In(1)EN, y B.*

mothers. This was accomplished by crossing sister females, homozygous or heterozygous for *dal*, by *Df(2L)J-der-39/Sco* males and comparing the recoveries of the *Sco* (*dal*/+) and the *M* (*dal*/$^-$) classes. These results, along with, for comparison, results of the same experiment involving *abo*, are shown in Table 8. It can be seen that for *dal*, as for *abo*, the probability of surviving the maternally induced abnormality in the egg is very much reduced in the absence of a wild-type allele of the maternal-effect gene.

A mapping of the da–abo *region*: The *da–abo* region is just proximal to the dominant marked *J*, which is at position 41 in the left arm of chromosome *2* on the standard genetic map of LINDSLEY and GRELL (1968) and at position 31DE on the standard salivary map (MANGE and SANDLER 1973). The most complete mapping of the *da–abo* region is shown in Figure 1. In the paragraphs to follow, the evidence for the details of this map are presented.

TABLE 8

The results of crosses of females carrying the indicated second chromosomes by males heterozygous for Df(2L)J-der-39 *and* Sco

Phenotype of progeny	Maternal second chromosomes			
	abo/Cy	*abo/abo*	*dal/Cy*	*dal/dal*
Cy Sco	740	—	1506	—
Cy + *(M)*	493	—	961	—
+ *Sco*	704	917	1233	1214
+ + *(M)*	379	89	293	38
Relative recovery*	0.18		0.13	

* The change in recovery of deficiency/mutant progeny relative to *Sco*/mutant progeny from homozygous mutant mothers relative to those same progeny recovered from heterozygous mothers. The *abo* data are from SANDLER (1975).

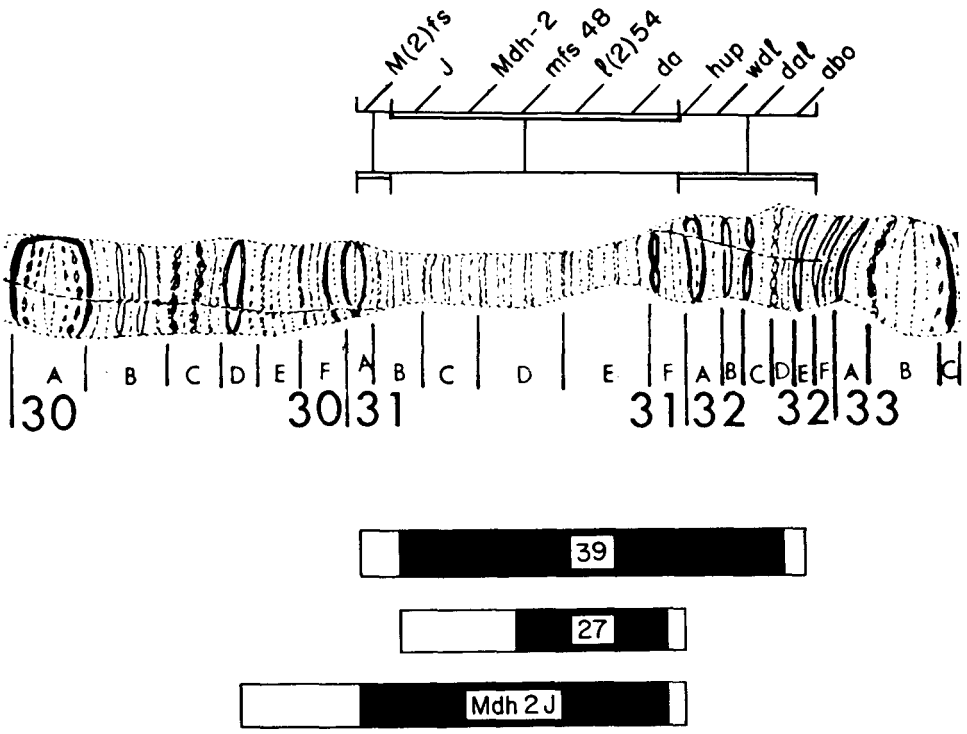

FIGURE 1.—A mapping of the *da-abo* region. The three deficiencies that primarily determine the mapping are shown below the chromosome with the cytological uncertainties of the breakpoints indicated by the clear parts of the bars. The evidence for the gene order (given above the chromosome) is discussed in text. The salivary-gland chromosome drawing is from KING (1970).

Genetically, *da* maps 0.5 units to the right of *J* (MANGE and SANDLER 1973), while the structural gene for *Mdh-2* maps 0.2 units to the right of *J* (GRELL 1969 and personal communication); this provides the indicated order, *J—Mdh-2—da*. In addition, *abo* maps 2.5 units to the right of *da* (SANDLER 1972), making *abo* the most proximal of these four elements.

LIFSCHYTZ and FALK (1969) have shown that radiation-reverted dominant mutations are frequently deletions that include the mutant locus. Accordingly, using the reversion of *J* as a selective procedure, five deficiencies that include the *J* locus have been X-ray induced and examined with respect to various of the elements in the region. The longest of the deficiencies, *Df(2L)J-der-39* (SANDLER 1975), includes most of salivary region 31 and the left half to two-thirds of region 32. Genetically, it contains a female-sterile Minute symbolized *M(2)fs*, *da*, *abo*, and the five new mutants reported here (that, of course, were selected as surviving poorly over this deficiency).

A second deficiency, *Df(2L)J-der-27*, is contained within *Df(2)J-der-39*. It does not include *M(2)fs*, *hup*, *wdl*, *dal*, or *abo*; it does include *J*, *mfs48*, *l(2)54*, and *da*. This provides the separation of *mfs(48)* and *l(2)54* from *abo*, *hup*, *wdl*,

and *dal*. No separation of *mfs48* and *l(2)54* from *da*, *J* or *Mdh-2* has been achieved; thus the order of the three genes between *J* and *da* in the figure is arbitrary.

The final deficiency pictured in the figure is *Df(2)Mdh2J* that was induced and tested by GRELL (1969). It is the only deficiency tested for the *Mdh-2* locus, and includes, in addition to it and *J*, *M(2)fs* and *da*. It has not been tested for the other loci. It is included here because it specifies the location of *M(2)fs* to the left of *J* and positions the *Mdh-2* locus, which may possibly mark the distal endpoint of the *da–abo* region.

To determine the relative positions of *hup*, *wdl*, *dal*, *da*, and *abo*, crosses were made between females carrying either *hup* or *wdl* on one chromosome *2* and a homolog carrying *da* and *abo* by *Df(2L)J-der-39/Sco* males. Sco^+-bearing progeny are almost certainly wild type for *da* and either *hup* or *wdl* (because these mutants survive so poorly over the deficiency)—but may be either *abo* or abo^+. There are three possible configurations in the test females. These are (using *x* for *hup* or *wdl*), + *da abo*/*x* ++; *da* + *abo*/+ *x* +; and *da abo* +/++ *x*. Therefore, da^+x^+ progeny will all be abo^+ if *x* is proximal to *da*. If *x* is between *da* and *abo*, they will all be *abo*. Finally, they may be either *abo* or abo^+ if *x* is distal to *abo*. In the case of *hup*, eight fertile Sco^+ male progeny were recovered and, upon progeny testing for *da*, *abo* and *hup*, all eight proved to be da^+, hup^+, and *abo*. In the case of *wdl*, eleven Sco^+ males were tested with the same result. It appears, therefore, most likely that *hup* and *wdl* are located between *da* and *abo*, and are so located in the figure. Because *dal* survives rather well when heterozygous with *Df(2L)J-der-39*, no further determination of its position was attempted. Therefore, the positions of *hup*, *wdl*, and *dal* in the figure relative to one another are arbitrary, as is the position of *dal* with respect to *abo*.

Two other deficiencies, *Df(2L)J-der-2* and *Df(2L)J-der-4*, for this region have been reported (MANGE and SANDLER 1973). They do not, however, add information to the map in Figure 1 and are, therefore, neither included in the figure nor considered further here.

DISCUSSION

Five different euchromatic autosomal maternal-effect mutations, all located within 2½ genetic units of one another and within 1½ numbered subdivisions on the standard salivary gland chromosome map and each exhibiting an interaction with sex-chromosome heterochromatin, have been characterized. It is suggested from this collection that these mutations—*da*, *hup*, *wdl*, *dal*, and *abo*—identify a special region of chromosome *2* that is defined by a cluster of genes, each of which interacts with sex-chromosome heterochromatin.

It is, to be sure, only intuitive that five mutants of this kind in a region this small identify a gene cluster; there are no data on the expected frequency of maternal-effect mutants that interact with heterochromatin and that should be found in a small region if such genes were randomly distributed on the chromosomes. There is, however, one observation on a maternal-effect mutant that causes sex-differential embryonic mortality but does not map in the *da-abo*

region; it does not interact with *Y*-chromosome heterochromatin. REDFIELD (1926) reported a second chromosome maternal-effect mutant, *l(2)mat*, that causes the death of female embryos such that 4–5 males per female are recovered among adult progeny. In experiments analogous to those reported in Table 3, we crossed *l(2)mat/l(2)mat* and *l(2)mat/In(2LR)Cy* sister females to males carrying an attached-*XY* chromosome and either no other sex chromosome or a *Y* chromosome. In the crosses involving *XY/O* males, the controls included 2,661 regular females and 3,715 regular males; the homozygous females produced 757 regular females and 4,449 regular males for a ♂-recovery of 4.2. In the crosses involving *XY/Y* males, the corresponding numbers were 1,533 and 1,593 in the control and 301 and 1,444 in the experimental, so that ♂-recovery was 4.6. Thus, with respect to interaction with a *Y* chromosome, *l(2)mat* does not behave like the five mutants in the *da-abo* region. A mapping indicated that *l(2)mat* was 16 units from *J*—clearly outside the *da–abo* region.

We now consider the comparative genetics of the five mutants identifying the *da–abo* region. They are all recessive, hypomorphic, maternal-effect mutations with no corresponding paternal effect. The consequence of the maternal effect in every case is less severe at 19° than it is at 25°. Three of the mutants are morphologically normal, one has upheld wings (*hup*) and one is *wd*-like (*wdl*).

The most striking similarity among these mutants is that they all interact with sex-chromosome heterochromatin—*Xh*, *Y*, or both. The best studied mutant in this set is *abo* (SANDLER 1970, PARRY and SANDLER 1974). For the case of *abo*, it has been shown, first, that homozygous mutant mothers produce defective eggs in the sense that such eggs often fail to hatch, and, second, that this egg defect can be partially compensated by a particular region of *Xh* (Xh^{abo} that is located in the distal penultimate one-eighth of the basal *X*-chromosome heterochromatin) and a (presumably) corresponding region on the *Y* chromosome. Thus, relative to abo^+ controls, zygotic survivals from homozygous *abo* mothers are: *XO* ♂♂ = 0.06; *XY* ♂♂ = 0.28; XX^- ♀♀ = 0.14; *XX* ♀♀ = 0.52, *XXY* ♀♀ = 0.72, illustrating both the rescuing effect of *Xh* and *Y* and the fact that females survive about twice as well as males when both carry the same amount of sex-chromosome heterochromatin.

It was possible to distinguish between the following two possibilities: (1) that *abo* and Xh^{abo} interact in some way important to the development of the egg such that the observed maternal effect is a consequence of a defective interaction, but that the defect in the egg can be partially compensated by supplying the zygote developing from an abnormal egg with extra doses of Xh^{abo}, and (2) that *abo* is involved in the packaging of Xh^{abo} or its product(s) into the egg so that the maternal effect is a consequence of defective packaging, with the properties of Xh^{abo} and the compensation by Xh^{abo} as just described. The distinguishing experiment was to compare the survival of *abo* and abo^+ zygotes produced from eggs laid by *abo* and abo^+ mothers. It was observed that *abo* zygotes of *abo* mothers survived only 50 percent as well as abo^+ zygotes of *abo* mothers or *abo* zygotes of abo^+ mothers (SANDLER 1970), while abo/abo^- zygotes from mutant mothers

survive only 18 percent as well as do *abo*$^+$ zygotes from the same mothers (SANDLER 1975 and Table 8). Because a gene for a packaging defect carried by a zygote should not affect the survival of that zygote, these results imply that *abo* interacts with Xh^{abo} in a way important for some aspect of the construction of normal eggs. This interaction also, evidently, can be important during development.

Although some of the tests have been somewhat indirect, it has been shown (SANDLER 1972; MASON 1973; MANGE and SANDLER 1973) that *da* has a set of properties implying that it, too, interacts with some aspect of sex-chromosome heterochromatin important in the production of normal eggs. Although Xh^{da} has not yet been mapped, it is most likely different from Xh^{abo} because, while the attached-*XY* chromosome behaves as an *X* plus a *Y* chromosome with respect to *abo* (that is, it appears to contain two doses of Xh^{abo}), it behaves as if it has but one Xh^{da} region.

In this report, we have shown that the three new mutants in the *da–abo* region—*hup*, *wdl*, and *dal*—also exhibit a set of properties suggesting that they interact with sex-chromosome heterochromatin in ways analogous to those of *da* and *abo*. While no attempt has yet been made to map the region of *Xh* that interacts with each of the new mutants, it seems likely that each of these is different, one from the others and each from Xh^{abo} and Xh^{da}. Thus, in the case of *hup*, that part of *Xh* deleted by $In(1)sc^{4L}sc^{8R}$ does not interact with the maternal effect, while the *Y* chromosome interacts lethally with it; in the case of *wdl*, *Xh* interacts lethally with the maternal effect, while the *Y* chromosome ameliorates it; with respect to *dal*, both *Xh* and *Y* partially compensate the maternal effect, as in the case of *da* and *abo*.

In this study, and in all previous reports on *da* and *abo*, only the consequences of variations in sex-chromosome heterochromatin have been examined. There is one experiment, however, performed by BARRY GANETZKY and JEFFREY HAEMER, that provides positive evidence of an interaction between the *abo*-induced maternal defect and autosomal heterochromatin. GANETZKY and HAEMER crossed wild-type (Canton-S) and homozygous *abo* females by males that were heterozygous for *In(2LR)Cy* and either a deficiency for heterochromatin from the right arm of chromosome *2*, $Df(2R)M\text{-}S2^{10}$, or from the left arm of chromosome *2*, *Df(2L)C'* (HILLIKER and HOLM 1975). In the case of $Df(2R)M\text{-}S2^{10}$, from *Canton-S* mothers, there were 3,831 *Cy* and 2,371 deficiency-bearing progeny recovered, while from *abo* mothers, the progeny included 1,701 *Cy* and 888 deficiencies. Therefore, from *abo* mothers, $Df(2R)M\text{-}S2^{10}$ is recovered only 0.84 as well as *In(2LR)Cy*. In the case of *Df(2L)C'*, from Canton-S mothers, the progeny included 5,342 *Cy* and 5,090 deficiencies, while the *abo* mothers produced 1,922 *Cy* and 2,657 deficiency-bearing progeny. Thus, *Df(2L)C'* is recovered 1.5 times as well as *In(2LR)Cy*.

$Df(2R)M\text{-}S2^{10}$ was X-ray induced and is likely deficient for most of the proximal heterochromatin of *2R* (LINDSLEY and GRELL 1968; HILLIKER and HOLM 1975). The result with *abo* just cited would suggest that included in this deficiency is a region ($2h^{abo}$) that interacts with *abo* in a way analogous to the inter-

action of *abo* and Xh^{abo}. *Df(2L)C'* was constructed by detaching compound-second chromosomes; it is the largest such deficiency extant (HILLIKER and HOLM 1975). It can be seen from this analysis that this method of producing a deficiency for *2L* heterochromatin necessarily results in a *2L*-deficiency-bearing chromosome that is simultaneously duplicated for an unknown amount of heterochromatin from *2R*. The results with *abo* noted here strongly suggest that the *2R* duplication carried by *Df(2L)C'* includes $2h^{abo}$ so that *Df(2L)C'*-bearing flies are triplicated for $2h^{abo}$. In addition, it appears that there is no similar region on *2L*.

Thus, although preliminary, it is likely that the specific heterochromatic sequence that interacts with any gene in the autosomal cluster is not necessarily restricted to one chromosomal position. This proposition is self-evidently intriguing in light of the distribution of satellite DNA's in *D. melanogaster* (PEACOCK *et al.* 1973).

There has been, to date, but one investigation into the nature of the interaction between heterochromatin and these autosomal genes. KRIDER and LEVINE (1975) showed (1) that in homozygous *abo* stocks, the *abo* effect gradually diminishes so that by generation 12 there is no maternal effect detectable by measuring the sex ratio in crosses to attached-*XY/O*-bearing males; and (2) that during this process, the number of rDNA cistrons on each of the *X* chromosomes in these stocks increases so that, by generation 12, each *X* chromosome has about seven times the number of rDNA cistrons that the *X* chromosomes of the original heterozygous stock had [apparently the *Y* chromosome remains unchanged (SANDLER 1975)]. The parallel with changes in the level of rDNA observed in other studies of these sequences in Drosophila (for a review, see TARTOF 1975), suggests that the Xh^{abo}-like elements may be composed of redundant cistrons with the *abo*-like elements controlling the level of redundancy. It is, however, not clear just why *abo* affects rDNA, since Xh^{abo} is not *NO*; several suggestions have been put forth by KRIDER and LEVINE (1975) and SANDLER (1975).

The general picture that emerges from the analyses of *da*, *abo*, *hup*, *wdl*, and *dal*, although still tentative and pregnant with possibilities, is that the basal heterochromatic region of each of the chromosomes of *D. melanogaster* contains some subset of a relatively small number of collections of repeated sequences. Whether or not these repeated sequences are the satellite DNA's themselves, something about them, perhaps a particular level of redundancy, is important in oogenesis. In the euchromatin of salivary regions 31–32 on the left arm of chromosome *2*, there is a set of regulator genes, each one regulating this property for one such sequence. It seems reasonable to imagine that these regulators are linked because they all act in development at the same time and in the same tissue—namely, during oogenesis and in the oocyte or associated (*e.g.*, nurse) cells.

It is, of course important to know whether the *da–abo* gene cluster is an example of a general tendency for genes having related functional roles in development to be linked, or whether the genes of higher organisms are, as at first glance they appear to be, distributed throughout the genome in a random fashion

with respect to developmental function, with the *da–abo* cluster representing a peculiarity. There are as yet no data to answer this question. An analog in Drosophila to the *da–abo* cluster might be the "bithorax complex," which has been very thoroughly studied by E. B. LEWIS, who has also discussed the general problem of gene clustering according to developmental function (LEWIS 1963, 1967).

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