ANALYSIS OF THE AUTOSOMAL MUTATION *abo* AND ITS INTERACTION WITH THE RIBOSOMAL DNA OF *DROSOPHILA MELANOGASTER*: THE ROLE OF *X*-CHROMOSOME HETEROCHROMATIN

BARRY YEDVOBNICK,* HALLIE M. KRIDER AND BRYAN I. LEVINE

Biological Sciences Group, Genetics and Cell Biology Section, University of Connecticut, Storrs, Connecticut 06268

Manuscript received October 15, 1979
Revised copy received March 20, 1980

ABSTRACT

The autosomal recessive, maternal-effect mutation abnormal oocyte (*abo*: 2–38) preferentially lowers the viability of *XO* progeny. The severity of the sex-ratio distortion is reduced by duplications of maternal or zygotic heterochromatin (SANDLER 1970, 1977; PARRY and SANDLER 1974). Utilizing *X*-chromosome inversions that contain modifications in the quantity and arrangement of the heterochromatic functions, Xh^{abo} and $cr+$, we have extended our investigations of *abo*'s influence on *XO* male recovery and rDNA redundancy (KRIDER, YEDVOBNICK and LEVINE 1979).——*XO* males bearing $In(1)sc^{s1L}sc^{4R}$ or $In(1)w^{m4}sc^{4R}$ are recovered twice as frequently as *X* chromosomes containing a single *Xh* region, implying that these inversions possess a duplication of Xh^{abo}. *abo* mutant females heterozygous for $In(1)sc^{s1L}sc^{4R}$ and wild-type *X* chromosomes generate *XO* progeny that do not contain elevated rDNA redundancies. *XO* males containing $In(1)w^{m4}$ exhibit male recoveries and rDNA elevations similar to those of males bearing a wild-type *X* chromosome, when both derive from a common *abo/abo* mother. Reciprocal crosses between $In(1)w^{m4}$ and Canton-S males to attached-*X* *abo* females show significant, though reduced, sex ratios in the absence of an rDNA effect. The observation that *abo* can elevate the rDNA redundancy of $In(1)w^{m4}$, a chromosome that does not compensate, suggests that *abo* and $cr+$ functions are not directly related.

THE chromosome *2* mutation, abnormal oocyte (*abo*:*2*–38), is a recessive maternal effector that reduces the viability of all progeny derived from mutant mothers, particularly *XO* males. SANDLER (1970) observed that the lethality imposed by *abo* was influenced by the amount of sex-chromosome heterochromatin present in mutant mothers or their progeny. This led SANDLER (1970) to suggest that the *abo* locus may interact with the ribosomal RNA cistrons (rDNA), which are situated within these heterochromatic regions.

KRIDER and LEVINE (1975) reported significant elevations in the rDNA content of homozygous *abo* lines. More recently, KRIDER, YEDVOBNICK and LEVINE (1979) demonstrated that the *XO* and *XXY* progeny of *abo/abo* mothers possess

* Present address: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511.

elevated rDNA redundancies, relative to the progeny of *abo*/+ controls. Apparently, the mutant *abo* locus is associated with unstable rDNA frequencies.

The compensatory response (cr^+) function has also been associated with the disproportionate replication of rDNA (PROCUNIER and TARTOF 1978). cr^+ has been localized to *X*-chromosome heterochromatin, distal to the nucleolus organizer (NO) region. PROCUNIER and TARTOF (1978) reported that, when "*cis* and contiguous" with the NO, cr^+ mediated the disproportionate replication of rDNA in *XO* and XX^{NO-} genotypes. cr^+ initiates a compensatory response at its adjacent NO region in the absence of an homologous cr^+, implying a *trans* interaction between cr^+ sites located on *X* or *Y* chromosomes.

The heterochromatic segment to which cr^+ is localized also has been implicated in *abo* phenotypic expression (PARRY and SANDLER 1974). This region, when duplicated in free fragments, reduces the viability effects of the *abo* mutation and is presumed to bear a site, Xh^{abo}, that interacts with the *abo* gene function. We speculated that if Xh^{abo} and cr^+ share a common chromosomal region, they may also represent a common function, a notion supported by the fact that both cr^+ and *abo* can influence rDNA redundancy. Therefore, we have attempted to extend the correlation between cr^+ and *abo*-mediated functions. In this study, we have (1) reexamined and confirmed PARRY and SANDLER's (1974) description of Xh^{abo} function, (2) separated the effect of *abo* on viability from its influence on rDNA redundancy, and (3) demonstrated that the interaction of *abo* with proximal *X* is unimpaired by a transposition of heterochromatin containing the Xh^{abo} and cr^+ functions. The latter observation shows that the *abo* influence on rDNA redundancy and viability is unchanged by a chromosomal configuration that eliminates the compensatory response. The major implication of our observations is that *abo* and cr^+ functions are not directly related.

MATERIALS AND METHODS

Drosophila stocks and culture conditions

D. melanogaster stocks were grown on cornmeal, agar and sucrose medium in bottles and vials at either 22 ± 1° or 25 ± 0.5°.

The following stocks were utilized in this study (for a complete description see LINDSLEY and GRELL 1968):

Canton-S: obtained from A. CHOVNICK (University of Connecticut) is the wild-type background of the *abo* mutation and represents the standard internal control for male recovery and rDNA redundancy analyses.

f166 Bowling Green: $sc^8YB^S/y\ sc^{S1}\ InS\ w^a sc^4$ and *y f:=*. The $In(1)sc^{S1L}sc^{4R}$ *X* chromosome is duplicated for most of the *X* heterochromatin, including the region reported to contain the Xh^{abo} and cr^+ loci (Figure 1).

f57 Bowling Green: Y^{bb} *su-var*/w^{m4}. The $In(1)w^{m4}$ chromosome breakpoint within *X* heterochromatin lies distal to the NO region, but proximal to the Xh^{abo} and cr^+ loci (Figure 1). Therefore, the latter loci in this rearrangement are no longer *cis*-contiguous with the NO region. The Y^{bb} *su-var* chromosome was substituted with a Canton-S *Y* chromosome in this study.

c6 Bowling Green: $y\ sc^4\ B\ f\ InS$, and *y f:=*. The $In(1)sc^4$ heterochromatic breakpoint is just proximal to the euchromatic-heterochromatic junction (Figure 1).

$In(1)w^{m4L}sc^{4R}$: This chromosome was selected as a recombination product from a female heterozygous for the $In(1)w^{m4}$ and $In(1)sc^4$ chromosomes. Males with appropriate markers

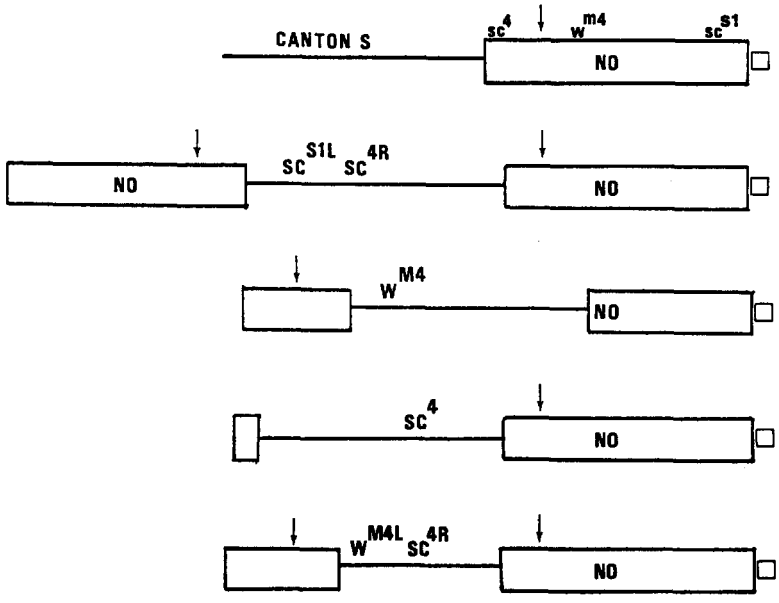

FIGURE 1.—Diagram of the wild-type (Canton-S) and inversion *X* chromosomes used in this study. Heterochromatin is represented as blocks, euchromatin as lines, and centromere as □. The arrow denotes the approximate location of the Xh^{abo}–cr^{+} region. NO = nucleolus organizer. For a detailed description of the breakpoints in these chromosomes, see LINDSLEY and GRELL (1968).

were selected and balanced against *C(1)RM/Y* females. Cytological analysis of mitotic neuroblast preparations from third-instar larvae confirmed that males of this stock bear *X* chromosomes with the distal heterochromatin of $In(1)w^{m4}$, and the proximal heterochromatin of $In(1)sc^{4}$ (Figure 1).

$\hat{XY}$ (*snoc*): Obtained from S. ABRAHAMSON is the following genotype: $Y^{S}XY^{L}$, *In(EN)ptg oc* sn^{5} and C(1)RM, *sc* ct^{n} sn^{X2} *ptg car*, *In(1)dl-49*, *y*/*O*. A spontaneous *w* mutation was isolated on the $\hat{XY}$ chromosome. Following matings to *XX* females, white-eyed males resulting from *X*-chromosome nondisjunction in females are readily scored. Male recovery values were corrected for nondisjunctions.

abo: In a Canton-S background and maintained in a heterozygous line opposite *In(2LR)Cy* was provided by L. SANDLER (University of Washington). The *Cy* chromosome possesses a wild-type *abo* locus. In the text, *abo/Cy* is referred to as *abo*/+.

$\hat{XX}$ abo: C(1)RM *y pn v* and $Y^{S}XY^{L}$, *In(1)EN*, *y B*/*O* was maintained against *In(2LR)Cy* and was also provided by L. SANDLER.

In the text, inversion *X* chromosomes will be referred to as $sc^{S1L}sc^{4R}$, w^{m4}, sc^{4} and $w^{m4L}sc^{4R}$. Furthermore, when these chromosomes are present in males without a *Y* chromosome (*XO* males), the genotype will be referred to as $sc^{S1L}sc^{4R}$ *XO*, w^{m4} *XO*, etc.

Generation of XO *progeny*: *XO* males were generated by 2 methods: (1) Females of the appropriate chromosome *2* genotype (*abo/abo*, *abo/Cy*) and carrying free *X* chromosomes were crossed to $\hat{XY}$ (snoc) males, and (2) Canton-S, w^{m4}, sc^{4}, and $w^{m4L}sc^{4R}$ males were crossed to $\hat{XX}$ *abo/abo*, *abo/Cy* females. Each male possessed a Canton-S *Y* chromosome. Since *XO* males are sterile, samples of the male progeny from the matings were crossed to virgin females and monitored for fertility. Only sterile lines were included in the male recovery data and only fertility-tested males were analyzed for rDNA content.

Male recovery values were calculated by the formula: Number of experimental males/control males × control females/experimental females (SANDLER 1977).

RNA-DNA hybridizations: The determination of rDNA redundancies in *XO* progeny was performed utilizing a microhybridization method recently developed in our laboratory (KRIDER, YEDVOBNICK and LEVINE 1979; YEDVOBNICK, KRIDER and DUTTON 1980). Since its initial description, the method has been simplified further by the omission of hydroxylapatite chromatography of hybrid molecules. We have reduced the experimental variation by directly binding duplexes to nitrocellulose filters (SCHLEICHER and SCHUELL) in 6 × SSC and carrier DNA (1 × SSC = 0.15 M NaCl, 0.015 M Na citrate).

The hybridization data presented in Tables 2, 6 and 7 derive from the analysis of at least 2 DNA isolations, with a minimum of 5 determinations performed on each DNA sample. Canton-S female DNA preparations were included as an internal control for all experiments.

XO hybridization values were corrected by a factor of 0.9 (TARTOF 1971). All values are ± standard error of the mean.

RESULTS

The effect of proximal X *duplications on the* abo *phenotype*: In their description of Xh^{abo}, PARRY and SANDLER (1974) utilized a series of *X*-chromosome duplications containing increasing amounts of heterochromatin to rescue the *XO* progeny of mutant females. Due to the diverse origins of these fragments (LINDSLEY and GRELL 1968), as well as the potential for position effects in this region (BAKER 1971), we initially attempted to confirm the existence of this function within *X*-chromosome heterochromatin.

Females heterozygous for the $sc^{s1L}sc^{4R}$ chromosome and a wild-type Canton-S *X* chromosome, and either *abo/abo*, *abo*/+ or +/+ were mated with $\hat{XY}$ males. The male recoveries of the two *XO* classes that derive from each mating are presented in Table 1. It is clear that *XO* males bearing the $sc^{s1L}sc^{4R}$ chromosome in a wild-type (+/+) background are recovered less than half as frequently as *XO* males containing the Canton-S *X* (1084 *vs.* 2751). However, the male recovery of the $sc^{s1L}sc^{4R}$ *X* chromosome derived from *abo/abo* relative to *abo*/+ females (0.45) is significantly greater than that of the Canton-S *X* (0.21). Clearly, the duplication of a proximal *X* region in *XO* males reduces the sex ratio imposed by *abo*. The rescue that results from the duplication of *Xh* in this experiment (0.45/0.21 = 2.14) is similar to that (2.41) observed by PARRY and SANDLER (1974), confirming their report of an Xh^{abo} function within proximal *X* heterochromatin.

We also examined whether an additional proximal *X*-region within mutant females influenced the effect of *abo* on the rDNA content of *XO* survivors. Pre-

TABLE 1

Results of the crosses of In(1)sc^{s1L}sc^{4R}, y w^a/*Canton-S*, ++ abo/abo, abo/+, +/+ *females* × $\hat{XY}$ *males at 22°*

	$\hat{XY}$	++ *XO*	++ *XO* recovery	*y w^a XO*	*y w^a XO* recovery
Control (+/+)	4268	2751		1084	
Control (*abo*/+)	3345	2368	0.21	1091	0.45
Experimental (*abo/abo*)	2964	448		437	

vious studies (KRIDER, YEDVOBNICK and LEVINE 1979) demonstrated that the progeny of sex ratio-expressing *abo/abo* mothers possess elevated rDNA redundancies.

The rDNA contents of the parental and progeny classes of Table 1 were measured (Table 2). It is apparent that the three classes of $sc^{S1L}sc^{4R}$/Canton-S females contain rDNA frequencies significantly greater than standard Canton S (0.383%) or Oregon-R (0.448%) lines, as expected for genotypes possessing an additional NO region. Furthermore, Canton-S *XO* males generated from each of these females contain approximately 20% more rDNA than predicted from the parental *X*-chromosome measurements. The percentage change of Canton-S *XO* males from *abo/abo* mothers (+18) is not greater than that of *XO* males derived from *abo/+* (+24) and +/+ (+16) control classes. Thus, there is no demonstrable *abo* effect on rDNA content beyond the 20% compensatory response.

$sc^{S1L}sc^{4R}$ *XO* males possess two NO regions and contain greater amounts of rDNA than Canton-S *XO* males, as expected. In addition, $sc^{S1L}sc^{4R}$ *XO*'s possess two cr^+ regions and, therefore, should not exhibit a compensatory response (PROCUNIER and TARTOF 1978). The percentage change of the $sc^{S1L}sc^{4R}$ *XO* males derived from *abo/abo*, *abo/+* and +/+ females is about half that observed from Canton-S *XO* males. Thus, the disproportionate replication of rDNA within the $sc^{S1L}sc^{4R}$ chromosome is much less than within Canton-S, especially when calculated on a per NO basis.

Furthermore, the percentage change of $sc^{S1L}sc^{4R}$ *XO* males from mutant females (+6) is lower than that of control classes (+12, +8). Thus, neither class of *XO*

TABLE 2

The rDNA content of XO *survivors from crosses of*

In(1)sc^{S1L}sc^{4R}, y w^a/*Canton-S*, ++ abo/abo, abo/+, +/+ *females* × $\hat{\text{XY}}$ *males*

Genotype	Percent rDNA	Percent change*
$sc^{S1L}sc^{4R}$/Canton-S +/+	0.669 ± 0.009	—
Canton-S *XO*	0.221 ± 0.008	+ 16
$sc^{S1L}sc^{4R}$ *XO*	0.517 ± 0.018	+ 8
$sc^{S1L}sc^{4R}$/Canton-S *abo*/+†	0.671 ± 0.009	—
Canton-S *XO*	0.245 ± 0.005	+ 24
$sc^{S1L}sc^{4R}$ *XO*	0.532 ± 0.006	+ 12
$sc^{S1L}sc^{4R}$/Canton-S *abo/abo*	0.727 ± 0.012	—
Canton-S *XO*	0.233 ± 0.006	+ 18
$sc^{S1L}sc^{4R}$ *XO*	0.560 ± 0.011	+ 6

* The percent change of the Canton-S *X* chromosome was calculated relative to the rDNA content of the chromosome used to generate the $sc^{S1L}sc^{4R}$/Canton-S parental female (0.191 +/+ class, 0.197 *abo/+*, *abo/abo* classes). The percent change of the $sc^{S1L}sc^{4R}$ chromosome was calculated relative to the difference between the $sc^{S1L}sc^{4R}$/Canton-S female and the Canton-S input values.

† Only non-Curly (*abo/+*) *XO* progeny were selected for the rDNA determinations.

males derived from $sc^{81L}sc^{4R}$/Canton-S females is subject to an *abo*-specific rDNA effect, although the recovery of both chromosomes is affected significantly by the *abo* mutation (Table 1).

Investigation of Xh^{abo} *and* cr^+ *functions:* In the w^{m4} *X* chromosome, cr^+ has been moved from a position *cis* and contiguous with the NO into euchromatin near the position of *w* (Figure 1). In this configuration, cr^+ does not mediate a compensatory response (PROCUNIER and TARTOF 1978). Utilizing this chromosome, we have compared the interaction of *abo* with Xh^{abo} and cr^+ functions by: (1) demonstrating that Xh^{abo} is transposed by w^{m4} and (2) determining whether the Xh^{abo} function or the influence of *abo* on *X*-chromosome rDNA content is affected by the transposition.

The position of Xh^{abo} *in* In(1)w^{m4}: Females heterozygous for the w^{m4} and sc^4 inversions were mated to Canton-S males, and recombinant $w^{m4L}sc^{4R}$ males were selected and confirmed cytologically. Based on the results of PARRY and SANDLER (1974), the sc^4 chromosome should possess a single Xh^{abo} function adjacent to the NO region, whereas $w^{m4L}sc^{4R}$ should contain an additional Xh^{abo} function within the distal w^{m4} heterochromatic segment.

sc^4 and $w^{m4L}sc^{4R}$ males were mated to $\hat{X}\hat{X}$ *abo/abo* and *abo*/+ females, and the male recovery of the *XO* progeny determined (Table 3). The sc^4 chromosome is subject to a significant *abo*-associated sex ratio, as *XO* males from mutant females are recovered only 37% as frequently as from controls. However, *XO* males bearing the $w^{m4L}sc^{4R}$ chromosome are recovered significantly more frequently (80%). The magnitude of rescue (0.800/0.373 = 2.14) in this experiment is identical to that observed in Table 1 (0.45/0.21 = 2.14).

We conclude from these data that a functional Xh^{abo} region is located in the inverted heterochromatin of w^{m4}. Consequently, the heterochromatic breakpoint of w^{m4} must lie proximal to Xh^{abo} and distal to the NO.

The effect of abo *on recovery and rDNA content of* w^{m4} *vs. Canton-S* XO *males:* We compared the effect of *abo* on the male recovery of w^{m4} and Canton-S *X* chromosomes by mating *abo/abo* and *abo*/+ females heterozygous for these chromosomes to $\hat{X}Y$ males. Since the expression of *abo* has been reported to be

TABLE 3

Results of the crosses of $\hat{X}\hat{X}$, y pn v/O abo/abo, abo/+ *females* × In(1)sc^4 *and* In(1)$w^{m4L}sc^{4R}$ *males at 22°*

		$\hat{X}^{\hat{}}XY$	*XO*	*XO* recovery
$\hat{X}\hat{X} \times sc^4$	Control	1948	1587	0.373
	Experimental	2807	852	
$\hat{X}\hat{X} \times w^{m4L}sc^{4R}$	Control	1245	789	0.800
	Experimental	2310	1171	

temperature sensitive (SANDLER 1970), these experiments were performed at 22° and 25°.

We observed a significant temperature effect on the male recoveries of the Canton-S and w^{m4} chromosomes; the recovery of both *XO* classes at 22° is more than twice that at 25° (Table 4). Furthermore, the recovery of w^{m4} XO males is not different from that of wild-type males (Table 4). Repeated determinations of Canton-S and w^{m4} *XO* male viability demonstrates that there is no significant difference in the mean male recovery values.

Results from the reciprocal matings, Canton-S and w^{m4} males to $\hat{XX}$ *abo/abo* and *abo/+* females, are presented in Table 5. *XO* males derived from these matings are subject to a significant temperature effect. Moreover, the male recoveries of wild-type and w^{m4} *X* chromosomes are identical at both temperatures. Therefore, the Xh^{abo} function persists when inverted in the w^{m4} inversion. We note that *XO* males are recovered in greater frequency from $\hat{XX}$ *abo/abo* females (Table 5) than from mutant females with free *X* chromosomes (Table 4), an observation previously reported by SANDLER (1970).

The rDNA content of the w^{m4} and Canton-S *XO* male progeny from the matings of Tables 4 and 5 are presented in Tables 6 and 7. As we have shown elsewhere (KRIDER, YEDVOBNICK and LEVINE 1979), Canton-S *XO* males derived from *abo/+* mothers do not compensate (+ 2%), whereas Canton-S *XO* males of *abo/abo* females possess elevated rDNA quantities (+ 12%, Table 6). This elevation is significant, but less than that observed previously. We note, however, that the absolute rDNA content observed in this experiment (0.221%) is very close to that reported earlier ,0.231%).

The rDNA frequencies in w^{m4} *XO* males from *abo/+* females are not greater than expected (−7%), as predicted from the observation of PROCUNIER and TARTOF (1978). However, those w^{m4} *XO* males surviving from mutant mothers possess 34% more rDNA than expected (Table 6). The rDNA elevation measured in w^{m4} indicates that the inversion of Xh^{abo} and cr^+ functions does not affect the interaction of *abo* with rDNA.

TABLE 4

Results of the crosses of In(1)w^{m4}*/Canton-S* abo/abo, abo/+ *females* × $\hat{XY}$ *males at 22° and 25°*

	$\hat{XY}$	w^{m4} *XO*	w^{m4} *XO* recovery	+ *XO*	+ *XO* recovery
Control (22°)	9302	2976	0.231	5358	0.240
Experimental (22°)	11364	839		1573	
Control (25°)	4023	2329	0.095	2355	0.112
Experimental (25°)	6228	344		410	

* Due to the poor viability of the w^{m4}; *Cy/+ XO* class at 22°, only w^{m4} *abo/+* and Canton-S *abo/+ XO* progeny were used to calculate the male recovery values at this temperature. The number of progeny in these classes was corrected by a factor of 2.

TABLE 5

Results of the crosses of $\widehat{XX}$, y pn v/O abo/abo, abo/+ *females* × *Canton-S and* In(1)w^{m4} *males at 22° and 25°*

		$\widehat{XXY}$	*XO*	*XO* recovery
$\widehat{XX}$ × Canton-S (22°)	Control	686	685	0.666
	Experimental	1871	1245	
$\widehat{XX}$ × w^{m4} (22°)	Control	366	348	0.667
	Experimental	795	504	
$\widehat{XX}$ × Canton-S (25°)	Control	469	587	0.383
	Experimental	741	355	
$\widehat{XX}$ × w^{m4} (25°)	Control	1273	1879	0.385
	Experimental	1620	920	

The rDNA redundancies of *XO* males derived from the reciprocal matings are presented in Table 7. In contrast to the results of Table 6 above, neither Canton-S nor w^{m4}*XO* males from *abo/abo* females possess an rDNA content significantly greater than that of *XO* males from *abo*/+ females.

DISCUSSION

The abnormal oocyte (*abo*) mutation was demonstrated by SANDLER (1970) to interact with sex-chromosome heterochromatin. Increased quantities of maternal or zygotic heterochromatic sequences resulted in marked elevations of progeny survival. A specific *X*-chromosome heterochromatic segment, Xh^{abo}, was later

TABLE 6

The rDNA content of XO *survivors from crosses of* In(1)w^{m4}*/Canton-S* abo/abo, abo/+ *females* × $\widehat{XY}$ *males*

Genotype	Percent rDNA	Percent change*
Canton-S/Canton-S *abo/abo* ♀	0.395 ± 0.014	—
w^{m4}/Canton-S *abo/abo* ♀	0.332 ± 0.014	—
w^{m4}/Canton-S *abo*/+ ♀	0.349 ± 0.020	—
Canton-S *XO via abo*/+ ♀†	0.202 ± 0.005	+ 2
Canton-S *XO via abo/abo* ♀	0.221 ± 0.004‡	+ 12
w^{m4} *XO via abo*/+ ♀†	0.141 ± 0.002	— 7
w^{m4} *XO via abo/abo* ♀	0.180 ± 0.007	+ 34

* Percent change is calculated relative to the parental starting values: 0.198% for the Canton-S chromosome and 0.134% (*abo/abo*), 0.151% (*abo*/+) for the w^{m4} chromosome. The Canton-S females measured above were used to generate the w^{m4}/Canton-S parental class.

† Only non-Curly (*abo*/+)*XO* progeny were selected for the rDNA determinations.

‡ Signigcantly higher than *XO* males *via abo*/+: $p < 0.01$ *t* test.

TABLE 7

The rDNA content of XO *survivors from crosses of* $\hat{X}\hat{X}$, y pn v, abo/abo, abo/+ *females* × *Canton-S and* In(1)w^{m4} *males*

Genotype	Percent rDNA	Percent change*
Canton-S ♀/♂	0.383 ± 0.01	—
w^{m4} ♂	0.331 ± 0.018	—
Canton-S *XO via*†		
$\hat{X}\hat{X}$ *abo*/+ ♀	0.209 ± 0.007	+ 9
Canton-S *XO via*		
$\hat{X}\hat{X}$ *abo/abo* ♀	0.214 ± 0.005	+ 12
w^{m4} *XO via*†		
$\hat{X}\hat{X}$ *abo*/+ ♀	0.144 ± 0.005	+ 3
w^{m4} *XO via*		
$\hat{X}\hat{X}$ *abo/abo* ♀	0.142 ± 0.006	+ 1

* Canton-S female and male rDNA contents have been measured independently and are identical. Therefore, the parental starting value for the Canton-S *X* chromosome in these experiments is 0.191%; the w^{m4} male possesses a Canton-S *Y* chromosome and its starting value was calculated as 0.140%.

† Only non-Curly (*abo*/+)*XO* progeny were selected for the rDNA determinations.

demonstrated to mediate the rescue of *XO* zygotes from mutant mothers (PARRY and SANDLER 1974).

We initiated this study by confirming the existence of Xh^{abo}. This was done for several reasons, initially to show that our *abo* stocks continue to express all previously reported functions. Furthermore, in their analysis of Xh^{abo}, PARRY and SANDLER (1974) utilized a series of X-ray-derived free duplications that bore increasing amounts of proximal *X* heterochromatin. Unfortunately, the actual disposition of the rDNAs, relative to the breakpoints, has not been characterized in these duplications. BAKER (1971), NIX (1973) and PUCKETT and SNYDER (1975) have presented evidence that rDNA function may be influenced by position-effects resulting from the juxtaposition of euchromatic sequences and heterochromatin containing the rDNAs. Thus, the possibility exists that PARRY and SANDLER's (1974) analysis demonstrated the distance from the rDNAs that a breakage event could be tolerated, rather than the discrete nature of Xh^{abo}. Similarly, the localization of Xh^{abo} could be subject to this source of error. That is, free *X* duplications, which apparently do not possess this function, actually may variegate at Xh^{abo}.

The $sc^{s1L}sc^{s4R}$ chromosome bears a duplication of the majority of proximal *X* heterochromatin. It is viable in *XO* males and bears seondary constrictions at both nucleolus organizers in mitotic neuroblast preparations of *XO* larvae (KRIDER and

PLAUT 1972; and repeated here) implying that neither rDNA complement is inactivated by the process used to generate the duplication. From the arguments of PARRY and SANDLER (1974), $sc^{S1L}sc^{4R}$ XO zygotes of *abo* mothers should survive significantly better, relative to controls, than *XO* zygotes bearing a normal *X* chromosome. It is clear from the data of Table 1 that our observations are consistent with this prediction; the elevation in male recovery is very close to that observed by those investigators. In addition, we see no evidence that breakage events near, and distal to the nucleolus organizer, diminish the viability of an *X* chromosome in the presence of the mutant *abo* allele. The male recovery of the w^{m4} *X* chromosome is not significantly lower than that of a wild-type *X* chromosome (Tables 4 and 5). Furthermore, the male recovery of the $w^{m4L}sc^{4R}$ chromosome is two-fold greater than that of sc^4 (Table 3), indicating that the inverted heterochromatin of w^{m4} contains an Xh^{abo} function. Thus, the w^{m4} heterochromatic breakpoint must be proximal to Xh^{abo} and distal to the NO.

Therefore, we conclude that we are able to demonstrate Xh^{abo} function in our *abo* stocks and that the analysis of Xh^{abo} is not complicated by position effects when the $sc^{S1L}sc^{4R}$, w^{m4} and $w^{m4L}sc^{4R}$ *X* chromosomes are employed. We argue that since position effects are probably not contributing factors in PARRY and SANDLER's (1974) analysis of Xh^{abo}, the site(s) responsible for Xh^{abo} function is discrete from the distal to the rDNAs, as they concluded.

The material recovered from the matings described above was analyzed for rDNA content. The rDNA redundancies of w^{m4} and Canton-S *XO* males derived from *abo*/+ females are not elevated (Table 6), in agreement with the results of PROCUNIER and TARTOF (1978) and KRIDER, YEDVOBNICK and LEVINE (1979). However, when these *X* chromosomes are recovered from *abo/abo* females, they possess rDNA frequencies significantly greater than their parental values (Table 6). The increase (34%) in rDNA of w^{m4} *XO* males from mutant mothers demonstrates that the interaction of *abo* with these cistrons is unimpaired by the inversion of heterochromatin containing the Xh^{abo} and cr^+ functions. More specifically, the rDNA effects of *abo* appear to be independent of the mechanism by which cr^+ drives the disproportionate replication of these cistrons, *i.e.*, the function that requires cr^+ to be contiguous with the NO. The ability of Xh^{abo} to function in w^{m4} and $w^{m4L}sc^{4R}$ suggests that it also is independent of this cr^+ function. However, we cannot rule out the possibility that the alternate cr^+ activity, that which interacts *trans* with additional cr^+ sites, contributes to Xh^{abo} function. PROCUNIER and TARTOF (1978) reported that this latter cr^+ function is expressed in the w^{m4} inversion.

We have observed two instances where *abo* does not influence rDNA quantities. The *XO* progeny derived from $sc^{S1L}sc^{4R}$/Canton-S females (Table 2), and from the $\hat{X}\hat{X}$ *abo* lines (Table 7) do not show *abo*-specific elevations in rDNA redundancy. In both cases, a significant sex ratio was evident (Tables 1 and 5) indicating that the viability effects of *abo* are separable from its effects on the rDNA cistrons. These results are not consistent with a model where *abo* simply selects for chromosomes bearing greater quantities of rDNA (KRIDER, YEDVOBNICK and LEVINE 1979).

It appears that the status of heterochromatin at numerous sites in the Drosophila genome can influence the phenotypic expression of *abo*. In addition to Xh^{abo}, *Y* chromosomes (SANDLER 1970) and portions of chromosome *2* heterochromatin (SANDLER 1977) interact with this mutation. In each case, the interaction was measured as an elevation in male recovery. Our results (Tables 1 and 3) and those of PARRY and SANDLER (1974) indicate that rDNA sequences are not directly responsible for zygotic rescue. Thus, the elevation of rDNA content in particular *XO* classes (Table 6) may result from the interaction of *abo* with other heterochromatic regions. For example, the activity of *abo* could be directed to a single site in *Xh* that initiates the replication of a number of adjacent sequences. This model predicts that only a subset of the amplified sequences could show rescuing activity. Alternatively, this locus may interact individually with an array of sequences dispersed throughout Drosophila heterochromatin, an idea considered previously by SANDLER (1977). The quantity of sex-chromosome and autosomal heterochromatin, as well as middle repetitive sequences such as the rDNAs, may be influenced by the *da-abo* region of chromosome *2*. The dissociation of the viability effects of *abo* from its rDNA effects may reflect the independent regulation of such sequences. We plan to focus future investigations on the satellite sequences that surround the NO region (PEACOCK *et al.* 1977).

Finally, it is significant that the 20% disproportionate replication of rDNA in the Canton-S *XO* progeny observed here (Table 2) is the first example of a compensatory response from this strain (MOHAN 1976; KRIDER, YEDVOBNICK and LEVINE 1979). Recentiy, we reported that the Canton-S *X* chromosome contains a cr^+ site, but does not compensate under conditions where disproportionate rDNA replication is observed in the Oregon-R strain (YEDVOBNICK, KRIDER and DUTTON 1980). The present results lead us to conclude that factors in addition to cr^+ and *abo* influence rDNA replication in the *XO* genotype and that such factors must be considered in any investigation of rDNA instability in this organism.

This research was supported by a Public Health Service Traineeship (B YEDVOBNICK), National Science Foundation grant PCM76-22728 and Public Health Service grant GM23605 to H. M. KRIDER. We thank PAMELA THOMAS for technical assistance.

LITERATURE CITED

BAKER, W. K., 1971 Evidence for position-effect suppression of the ribosomal RNA cistrons in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **68**: 2472–2476.

KRIDER, H. M. and B. I. LEVINE, 1975 Studies on the mutation abnormal oocyte and its interaction with the ribosomal DNA of *Drosophila melanogaster*. Genetics **81**: 501–513.

KRIDER, H. M. and W. PLAUT, 1972 Studies on nucleolar RNA synthesis in *Drosophila melanogaster*. II. The influence of conditions resulting in a bobbed phenotype on rate of synthesis and secondary constriction formation. J. Cell Sci. **11**: 689–697.

KRIDER, H. M., B. YEDVOBNICK and B. I. LEVINE, 1979 The effect of *abo* phenotypic expression on ribosomal DNA instabilities in *Drosophila melanogaster*. Genetics **92**: 879–889.

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

MOHAN, J., 1976 Ribosomal DNA and its expression in *Drosophila melanogaster* during growth and development. Molec. Gen. Genet. **147**: 217–223.

NIX, C. E., 1973 Suppression of transcription of the ribosomal RNA cistrons of *Drosophila melanogaster* in a structurally rearranged chromosome. Biochem. Genet. **10**: 1–12.

PARRY, D. M. and L. SANDLER, 1974 The genetic identification of a heterochromatic segment on the *X* chromosome of *Drosophila melanogaster*. Genetics **77**: 535–539.

PEACOCK, W. J., A. R. LOHE, W. L. GERLACH, P. DUNSMUIR and R. APPELS, 1977 Fine structure and evolution of DNA in heterochromatin. Cold Spring Harbor Symp. Quant. Biol. **42**: 1121–1135.

PROCUNIER, J. D. and K. D. TARTOF, 1978 A genetic locus having *trans* and contiguous *cis* functions that control the disproportionate replication of ribosomal RNA genes in *Drosophila melanogaster*. Genetics **88**: 67–79.

PUCKETT, L. D. and L. A. SNYDER, 1975 Biochemical evidence for position-effect suppression of ribosomal RNA synthesis in *Drosophila melanogaster*. Exp. Cell Res. **95**: 31–38.

SANDLER, L., 1970 The regulation of sex chromosome heterochromatic activity by an autosomal gene in *Drosophila melanogaster*. Genetics **64**: 481–493. ——, 1977 Evidence for a set of closely linked autosomal genes that interact with sex chromosome heterochromatin in *Drosophila melanogaster*. Genetics **86**: 567–582.

TARTOF, K. D., 1971 Increasing the multiplicity of ribosomal RNA genes in *Drosophila melanogaster*. Science **171**: 294–297. ——, 1974 Unequal mitotic sister chromatid exchange as the mechanism of ribosomal RNA gene magnification. Proc. Natl. Acad. Sci. U.S. **71**: 1272–1276.

YEDVOBNICK, B., H. M. KRIDER and F. L. DUTTON, 1980 Analysis of disproportionate replication of ribosomal DNA in *Drosophila melanogaster* by a microhybridization method. Biochem. Genet. (in press).

Corresponding editor: L. SANDLER