THE REGULATION OF SEX CHROMOSOME HETEROCHROMATIC ACTIVITY BY AN AUTOSOMAL GENE IN *DROSOPHILA MELANOGASTER*[1]

L. SANDLER

Department of Genetics, University of Washington, Seattle, Washington 98105

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A second chromosome extracted from a natural population of *Drosophila melanogaster* has the property that females homozygous for that chromosome and carrying normal *X* chromosomes—when crossed to males carrying an attached-*XY* chromosome, no other sex chromosomes, and normal second chromosomes—produce a large excess of female offspring (SANDLER *et al.* 1968). The mutant responsible for the aberrant sex ratio, carried by this chromosome *2*, has been named "abnormal oocyte" (symbol: *abo*). The sex-ratio effect of homozygous *abo* is shown in Table 1; the parental females, whose progeny are recorded, were recovered from crosses of *+/In(2LR)Cy* males and females and from crosses of *abo/In(2LR)Cy* males and females. These data also show that the mutant is completely recessive and that the abnormal sex ratio is not the result of differential mortality caused by heterozygosity for *abo* in the progeny.

An abnormal sex ratio owing to an autosomal gene in the parental female (the homogametic sex) when, as in the present case, not the result of sex reversals (as can be seen from the progeny phenotypes) admits of only two possible explanations: either the two sexes are produced with different frequencies or they die differentially. That is, either (a) homozygous *abo* females control the relative frequency with which their eggs are fertilized by *XY*-bearing as opposed to 0-bearing sperm which would suggest that females can distinguish *X*-bearing from *Y*-bearing sperm and, by controlling the relative frequency of fertilization through the action of genes such as *abo*$^+$, be responsible for adjusting the primary sex ratio, or (b) *abo/abo* females produce eggs abnormal in respect of a matern-

TABLE 1

Results of crosses of females with normal, unmarked X *chromosomes and with the indicated chromosome* 2 *constitution by* $Y^sX{\cdot}Y^L$, In(1)EN, y B/0 *males*

Constitution of parental female	Regular progeny		Exceptional progeny		Ratio
	B♀♀	+♂♂	+♀♀	*y B*♂♂	(*B*♀♀/Regular progeny)
+/+	3166	4153	0	1	0.43
+/In(2LR)Cy	3406	4628	1	9	0.42
abo/In(2LR)Cy	3539	4900	1	7	0.42
abo/abo	7276	983	5	24	0.88

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ally-produced substance, an abnormality which in these experiments causes *X0* males to die more frequently than *XXY* females which would suggest that *abo*$^+$ controls the amount, distribution or nature of some maternal substance in the oocyte necessary for normal survival of the zygote.

Evidence will be presented indicating that the latter proposition is the correct one, and in particular that *abo*$^+$ is a gene, active throughout the life cycle, that controls either the expression of structural genes located in the basal heterochromatin of the sex chromosomes or the activity or stability of their products. Because the cistrons for ribosomal RNA are known to be located in the sex chromosome heterochromatin (RITOSSA and SPIEGELMAN 1965), because (other than male-fertility factors) this is the only known heterochromatic function, and because there is direct evidence in Drosophila for factors outside the sex chromosome heterochromatin that control rRNA in eggs (SCHULTZ and TRAVAGLINI 1965), it is suggested that *abo*$^+$ is a component in the regulation of the quantity of cellular rRNA.

ELEMENTARY GENETICS OF *abo*

The origin and isolation of the *abo*-bearing second chromosome is described in detail in SANDLER *et al.* (1968); it was recovered as a sex-ratio mutant from a natural population near Rome, Italy. Homozygous *abo* individuals, of either sex and from either heterozygous or homozygous *abo* parents, exhibit no morphological abnormalities. Since, as noted above, *abo* may be implicated in the control of the quantity of rRNA, especial attention was given to phenotypic effects such as those of the bobbed and Minute loci; none, however, was observed.

The original chromosome *2* isolated from nature has been put into a stock with all the other chromosomes replaced by chromosomes from a standard, Canton-S stock and been allowed two generations of free recombination with the Canton-S second chromosome followed by reisolation of *abo*. Then a stock entirely Canton-S, except for the *abo* locus and some unknown amount of the original chromosome *2*, balanced with the multiply-inverted, dominantly-marked, recessive lethal chromosome, *In(2LR)Cy*, was constructed; this stock is used as the source of *abo* in all the experiments to be reported. Because *abo* is completely recessive, *abo/In(2LR)Cy* individuals are generally used as controls.

Salivary gland preparations show that the *abo*-bearing chromosome is not associated with any euchromatic aberrations; crossing over between *pr* and *cn* (which are about one unit apart and span the centromere) is the same in *abo/pr cn* and *+/pr cn* heterozygotes, indicating the absence of gross heterochromatic rearrangements. The *abo* locus has been mapped by collecting a random sample of second chromosomes from *abo/pr cn* females (and also selecting a number of *pr cn* chromosomes and recombinants between *pr* and *cn*) and analyzing these. The random sample of chromosomes included: 52 + *pr cn*; 44 *abo* + +; 4 *abo pr cn*; 16 + + +; and 1 *abo* + *cn*. The selected *pr cn* and *pr–cn* recombinants were: 22 + *pr cn*; 3 *abo pr cn*; 6 + *pr* +; and 5 *abo* + *cn*. Thus *abo* is in the euchromatin of the left arm of chromosome *2* at about position 38 on the standard map.

It will be noted in Table 1 that in addition to the abnormal sex ratio conditioned by homozygous *abo*, there is a somewhat elevated frequency of primary nondisjunction; the comparison is with the +/+ females, as *In(2LR)Cy* itself increases *X* nondisjunction. In addition, in the original tests of *abo* (SANDLER *et al.* 1968), three intersexes (the products of unreduced eggs) were noted among a small number of progeny. These observations suggest that either *abo* itself or another gene on the *abo*-bearing chromosome is a nondisjunction-producing meiotic mutant. In the crossover tests described above, nondisjunction was not highly correlated with *abo*; it is probable, therefore, that *abo* is not the meiotic mutant; but the evidence is weak because the frequency of nondisjunction is so low. Nevertheless, we will not, in this report, consider the problem of nondisjunction further and will base all calculations on the regular progeny only; this will not, of course, materially affect any of the conclusions reached.

Finally *abo* was detected and mapped by its effect on the sex ratio among the progeny of homozygous females. No analogous effect is found in the progeny of homozygous males. Because, as will be shown below, the sex chromosome constitution of the progeny of homozygous *abo* females influences the sex ratio, the effect of *abo* in males was examined in a variety of crosses. The crosses and results are given in Table 2.

In summary, then, *abo* is a recessive, euchromatic, point mutant, located at about 38 on chromosome *2*, with no visible effects. When homozygous in otherwise normal females, it results in the production of a large excess of female progeny in crosses to homozygous *abo*$^+$ males carrying an attached-*XY* chromosome and no free *Y*. It has no obvious effect of any kind in males.

NATURE OF THE SEX-RATIO EFFECT

As already noted, the abnormal sex ratio conditioned by *abo/abo* females could be the result either of preferential fertilization by *XY*-bearing sperm or a greater mortality among *X0* sons than among *XXY* daughters. To examine these two

TABLE 2

Results of crosses of males carrying normal, unmarked, sex chromosomes and either abo/In(2LR)Cy *(Control) or* abo/abo *(Exp.) by females with the indicated sex chromosome constitution*

Constitution of parental female		Progeny ♀♀	♂♂	Ratio (♀♀/total)
y/y	Cont.	3256	3296	0.50
	Exp.	3026	3176	0.49
C(1)DX,y f/Y*	Cont.	1176	1680	0.41
	Exp.	1138	1470	0.44
C(1)RM,y pn v/0	Cont.	1801	1926	0.48
	Exp.	1505	1381	0.52

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

possibilities, females carrying normal, unmarked *X* chromosomes and either heterozygous or homozygous for *abo* were crossed to homozygous *abo*$^+$ males of four different sex chromosome constitutions: (1) *X-Y/0*; (2) *X-Y/Y*; (3) *X/Y*; and (4) *X/Y* where the *X* chromosome carried the heterochromatic recessive, *bb*. The results of these crosses are given in the upper part of Table 3 along with a measure (♂ recovery) of the effect of *abo/abo* relative to the *abo/In(2LR)Cy* controls.

From the first three crosses, a sample of eggs was counted and the adults recovered from them scored. These results, along with summaries of ♂ recovery, total survival and survival by sex are given in the lower part of Table 3.

From these results, four conclusions emerge. First, the sex ratio conditioned by homozygous *abo* in the parental female varies widely according to the sex chromosome constitution of the parental male. Secondly, the abnormal sex ratios are the result of differential zygotic survival. What is especially striking in this connection is that although the sex ratio is different in each cross, an individual with a particular zygotic constitution has a constant probability of survival: thus *XXY* females survived 73% as well as in the control in crosses to *X-Y/0* males and 71%

TABLE 3

Results of crosses of females with normal, unmarked X chromosomes carrying either abo/In(2LR)Cy*(Control) or* abo/abo *(Exp.) by either (1)* $Y^sX{\cdot}Y^L$, In(1)EN, y B/0 *males; (2)* $Y^sX{\cdot}Y^L$, In(1)EN, y B/Y *males; (3)* B/Y *males; or (4)* B bb/Y *males*

	X-Y/0		*X* constitution of parental male *X-Y/Y*		*B/Y*		*B bb/Y*	
Classes	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
Regular ♀ ♀	5383	3706	5616	4302	6117	3269	5562	2258
Regular ♂ ♂	5665	369	5481	2009	6592	2552	5931	2044
Exceptional ♀ ♀	5	4	0	9	3	8	2	12
Exceptional ♂ ♂	4	13	6	8	3	7	1	4
♂ recovery*		0.09		0.48		0.72		0.85
Total eggs	586	552	955	759	583	735	....	....
Regular ♀ ♀	281	190	490	257	270	170	....	....
Regular ♂ ♂	284	17	412	104	293	114	....	....
Exceptional ♀ ♀	0	0	0	0	0	0	....	....
Exceptional ♂ ♂	1	1	0	1	0	0	....	....
♂ recovery*		0.09		0.48		0.62		
Adult survival	0.97	0.38	0.94	0.48	0.97	0.39	....	....
♀ survival†		0.73		0.71		0.50		0.35
♂ survival†		0.06		0.30		0.31		0.30

* ♂ recovery = observed number of regular males in the experimental cross divided by the number of males that would have appeared relative to the regular females if the sex ratio in the experimental cross were that in the corresponding control.

† survival by sex is computed from the observed number of that sex in the experimental egg-counted set divided by an expectation calculated from the adult recovery in the egg-counted control and the sex ratio among the regular progeny in the overall control (i.e., the upper section of the table).

as well in crosses to *X-Y/Y* males, while *XY* males survived 30% and 31% as well as in the controls—the comparison here being between the crosses to *X-Y/Y* and *X/Y* males. It might be noted that the mortality measured here occurs in the egg stage: in the cross to *X-Y/0* males, in a sample of 100 fertilized eggs from control females, 99 hatched within 48 hours while only 44 of 100 eggs from homozygous *abo* mothers did so. Thirdly, survival in either sex is increased by the addition of a *Y* chromosome to the zygote. Thus the survivals, relative to the controls, were: *XXY*♀♀ = 72%; *XX*♀♀ = 50%; *XY*♂♂ = 30%; *X0*♂♂ = 6%. Finally, there appears to be an effect, analogous to that of the *Y* chromosome, of the basal heterochromatin of the *X* chromosome. It can be seen that the sex ratio in the crosses to *B bb/Y* males is significantly different from that observed in crosses to *B bb*$^+$*/Y* males. Although no egg counts were made in the *bb* crosses, the male offspring are identical with those from the *X-Y/Y* and *B bb*$^+$*/Y* crosses and may therefore be assumed to have a relative survival of 30%; under this assumption, *B bb/++* females from homozygous *abo* mothers survive 35% as well as in the control compared to 50% for homozygous *bb*$^+$ females. These *bb/+* females, it may be noted, are phenotypically *bb*$^+$. Since the *bb* locus is located in the heterochromatin of the *X* chromosome, has an allele on the *Y*, and the mutant, *bb*, is likely associated with a partial deficiency of the heterochromatin (RITOSSA, ATWOOD and SPIEGELMAN 1966), it seems reasonable to attribute the effect of *bb* in *abo* crosses to this heterochromatic deficiency.

Additional evidence that this is, in fact, the case is provided by the following experiment involving a paternal *X* chromosome containing a large heterochromatic deficiency. From a cross of *y/y, abo/In(2LR)Cy* females by *y/Y; abo/In (2LR)Cy* males, homozygous *y* females, either homozygous or heterozygous for *abo*, were recovered and crossed (a) to $Y^{S}X{\cdot}Y^{L}$, *In(1)EN, y B/0* males to control the magnitude of the sex-ratio effect after substitution of the *X* chromosomes, and (b) to *In(1)sc*^{4L}*sc*^{8R}, *y cv v f/y*$^+$*Y* males. The paternal males of both types were homozygous *abo*$^+$. The results of the crosses are given in Table 4. It can be seen from the ♂ recovery value (given in the footnote to Table 4) calculated from the crosses to *X-Y/0* males, that the *abo* effect is not influenced by the substitution of the *X* chromosomes (*y* for +).

In the *sc*^{4}*sc*^{8} crosses, the parental males carry an *X* chromosome deficient for almost all of the basal heterochromatin including the *bb* locus. Because this deficiency also includes the region of homology with the *Y* chromosome, sex chromosome disjunction is very irregular; that is, significant numbers of sperm are produced carrying either the deficient *X* chromosome only or the *Y* chromosome only (the regular classes), and also both sex chromosomes or neither sex chromosome. Thus we may, in this experiment, examine the effect of homozygous *abo* in the parental female on the viability of *abo/+* progeny that carry: a normal *X* and a deficient *X*; a normal *X* and *y*$^+$*Y*—which, in these experiments, may not be directly comparable to a normal *Y* chromosome because the *y*$^+$*Y* has an unknown amount of *Y* chromosome heterochromatin replaced by *X* chromosome heterochromatin (for the origin of both *In(1)sc*^{4L}*sc*^{8R} and *y*$^+$*Y*, see LINDSLEY

TABLE 4

The results of crosses of females, homozygous for the sex-linked recessive y *and either* abo/abo *(Experimental) or* abo/In(2LR)Cy *(Control)*, by homozygous* abo+ *males carrying, in addition,* In(1)sc^{4L} sc^{8R}, y cv v f/y+ Y†

Cross		Contribution of sperm (and phenotype of progeny) $X(y\,♀\,♀)$	$Y(+\,♂\,♂)$	$X+Y(+\,♀\,♀)$	$0(y\,♂\,♂)$
Control	Number	3486	1771	149	1820
	Percent	48	25	2	25
Experimental	Number	878	773	140	194
	Percent	44	39	7	10

* Crosses of the same females by $Y^{S}X{\cdot}Y^{L}$, *In(1)EN*, *y B/0* males gave a ♂ recovery value, computed as in Table 3, of 0.09 based on 2462 regular ♀ ♀ and 3083 regular ♂ ♂ in the *abo/In(2LR)Cy* control and 1973 regular ♀ ♀ and 229 regular ♂ ♂ in the experimental set.

† Primary nondisjunction in the parental female can be recognized in these crosses, but only when nullo-*X* eggs are fertilized by $(X + Y)$-bearing sperm resulting in *cv v f* ♂ ♂; none was observed in either the experimental or control crosses.

and GRELL 1968); a normal *X*, a deficient *X* and $y^{+}Y$; and *X0* males carrying a normal *X* chromosome. By comparing complementary classes, it can be seen that, as before, *X0* males are recovered from homozygous *abo* mothers with a much lower frequency than in the control; in addition, and more importantly, there is a drastic reduction in the recovery of X/X^{def} females (the y ♀ ♀). Thus the hypothesis that *X* chromosome heterochromatin will compensate the *abo*-induced effect is confirmed.

A comparison with the previous results (i.e. those of Table 3) is possible if we assume that in these experiments, the *X0* male progeny (y ♂ ♂) of homozygous *abo* mothers have the same relative viability as before (6%). In that case, the survivals of the other classes are: X/X^{def} ♀ ♀ = 14%; $X/y^{+}Y$ ♂ ♂ = 25%; and $X/X^{def}/y^{+}Y$ ♀ ♀ = 53%.

In summary, then, it is clear that zygotes developing from eggs produced by homozygous *abo* females survive less well than do genetically identical zygotes from eggs from normal (*abo*/+ or +/+) females; the reduced survival can, however, be partially compensated for by additional sex chromosome heterochromatin in the developing zygote. This suggests that the effect of *abo* concerns a maternal substance in the oocyte (and not sperm selection) necessary for zygotic survival, which is deficient or abnormal in eggs from *abo/abo* mothers, but which can be compensated for by some product of the sex chromosome heterochromatin which is produced after fertilization.

Although this seems to be the most reasonable interpretation of the results, three problems present themselves immediately. (1) While the aberrant sex ratios are demonstrably the result of differential mortality, the effect might still be fundamentally sperm selection—the selection being the production of unfertilized eggs (or zygotes destined to die for any reason) with different frequencies for the different sperm types, and with the nature of the sperm preferences

being responsible for the correlation of recovery and the sex chromosome constitution of the zygote. (2) Since the amount of sex chromosome heterochromatin in the zygote influences the sex ratio, it is conceivable that the sex chromosomes carried by the *abo/abo* mother herself are also important. (3) It has been seen that *XX* females survive better than *XY* males (50% *vs.* 30%); it would seem, therefore, that either there is a sex effect superimposed on the effect of heterochromatin or else that the *X* and *Y* chromosomes are not equivalent in their capacity to compensate for the *abo*-induced abnormality. More generally, it is clear from all of the data that the probability of survival is not related to dosage of sex chromosome heterochromatin in any mathematically simple way.

To examine these questions, two additional experiments have been performed. First, heterozygous and homozygous *abo* females carrying an attached-*X* chromosome and no *Y* chromosome (from a cross of *C(1)RM, y pn v/0; abo/In(2LR)Cy* ♀♀ × $Y^sX{\cdot}Y^L$, *In(1)EN, y B/0; abo/In(2LR)Cy* ♂♂) or carrying a Y chromosome (from a cross of *C(1)RM, y pn v/0; abo/In(2LR)Cy* ♀♀ × *+/Y; abo/In(2LR)Cy* ♂♂) were crossed with homozygous *abo*+ males of three different sex chromosome constitutions: (1) $Y^sX{\cdot}Y^L$, *In(1)EN, y B/0;* (2) $Y^sX{\cdot}Y^L$, *In(1)EN, y B/Y*; and (3) *B/Y*. The results of these crosses are presented in Table 5.

Secondly, from a cross of +/+; *abo/In(2LR)Cy* females by $Y^sX{\cdot}Y^L$, *In(1)EN, y B/0; abo/In(2LR)Cy* males, heterozygous and homozygous *abo* females carrying the attached-*XY* chromosome and a normal *X* were recovered and crossed to

TABLE 5

Results of crosses of abo/abo *(Exp.) or* abo/In(2LR)Cy *(Cont.) females with the indicated sex-chromosome constitutions by* abo+/abo+ *males with the indicated sex-chromosome constitutions*

Constitution of parental female	Constitution of parental male		♀♀	♂♂	♂ recovery
C(1)RM,y pn v/0	$\overline{XY}$,*y B/0*	Cont.	1390	1098	1.00
		Exp.	2663	2112	
C(1)RM,y pn v/0	$\overline{XY}$,*y B/Y*	Cont.	934	1173	0.72
		Exp.	2579	2342	
C(1)RM,y pn v/0	*B/Y*	Cont.	1057	1226	0.47
		Exp.	2121	1314	
C(1)RM,y pn v/Y	$\overline{XY}$,*y B/0*	Cont.	1484	1326	0.97
		Exp.	3859	3348	
C(1)RM,y pn v/Y	$\overline{XY}$,*y B/Y*	Cont.	1389	1433	1.00
		Exp.	3968	4104	
C(1)RM,y pn v/Y	*B/Y*	Cont.	1366	1516	0.85
		Exp.	4045	3801	

"♂ recovery" is calculated as in Table 3.

$Y^sX{\cdot}Y^L$, *In(1)EN, v f B/0;* abo^+/abo^+ males. The results of these crosses are given in Table 6.

The possibility of *abo* being a gene influencing sperm selection is definitively eliminated by the results of the crosses with attached-*X/0* females (the upper section of Table 5). There it can be seen that relative recovery is, as before, positively correlated with the amount of sex chromosome heterochromatin in the zygote; now, however, the sex chromosome constitution of the sperm types giving rise to the classes are all reversed relative to the experiments with free-*X*-bearing females. This reversal is true in each experiment individually and also among experiments. Thus, on the hypothesis of sperm selection, the selection preferences inferred from the free-*X* crosses (Table 3) would have been $X\text{-}Y > X > Y > 0$; with attached-*X*'s, however, the preferences would have to be $Y > X\text{-}Y = 0 > X$.

While sperm selection can be eliminated by these data, the general question of the magnitude of the effect is further complicated. The comparison of results of attached-*X* and free-*X* crosses in terms of ♂ recovery (the ♂ recovery values are computed from Tables 3 and 5) is:

$$\overline{XX}\,♀♀ : X\text{-}Y\,♂♂ = 1.00 \textit{ vs. } XX\,♀♀ : XY\,♂♂ = 0.72$$
$$\overline{XXY}\,♀♀ : X\text{-}Y\,♂♂ = 0.72 \textit{ vs. } XX\text{-}Y\,♀♀ : XY\,♂♂ = 0.48$$
$$\overline{XXY}\,♀♀ : X0\,♂♂ = 0.47 \textit{ vs. } XX\text{-}Y\,♀♀ : X0\,♂♂ = 0.09.$$

It is clear, therefore, that with respect to the *abo*-induced effect, an attached-*X* and a *Y* are not the same as two free *X*'s and a *Y*, and an attached-*XY* is different from a free *X* and *Y*. However, in addition, the magnitude of the effect appears generally lower in the attached-*X* crosses. An explanation that suggests itself is that the same process that results in compensation of the *abo*-induced effect in the zygote owing to additional sex chromosome heterochromatin is also operative in the *abo/abo* mother; that is, that the more sex chromosome heterochromatin the

TABLE 6

Results of crosses of +/$Y^sX{\cdot}Y^L$, In(1)EN, y B *females carrying either* abo/In(2LR)Cy *(Control) or abo/abo (Experimental) by* $Y^sX{\cdot}Y^L$ In(1)EN, v f B/0 *males*

Type of progeny	Phenotype	Control	Experimental	Relative recovery*
X-Y/X-Y	*B/B* ♀♀	487	637	1.75
X/X-Y	*B/+* ♀♀	842	828	1.32
X-Y/0	*y B* ♂♂	988	732	0.99
X/0	+ ♂♂	1211	438	0.48
Crossover	*y* ♂♂	60	25	...
	B ♂♂	72	40	...
Patroclinous	*v f B* ♂♂	86	67	...

* The recovery of the indicated class in the experimental set relative to the control computed from the regular, noncrossover classes only.

homozygous *abo* mother has, the less abnormal will be the eggs she produces. Under this simplifying hypothesis, it is necessary to imagine only that the attached-*X* chromosome contains more heterochromatin than two free *X*'s. Any attached-*X* will, almost necessarily, have an amount of heterochromatin different from two free *X*'s, but it is very difficult to determine the direction and the extent of the difference because of the absence of heterochromatic markers (for a general description of the origin of attached-*X*'s, see LINDSLEY and GRELL 1968). The general idea proposed here, however, can be examined in two ways. First, the *abo* effect from females carrying this same attached-*X* and a *Y* chromosome (lower part of Table 5), should be, and is, reduced compared with the effect produced by attached-*X* females carrying no free *Y*. Secondly, an examination of the progeny of *X/X-Y* females (Table 6) further confirms the suggestion. In this cross, as in all the others, relative recovery (survival) is positively correlated with the amount of sex chromosome heterochromatin in the zygote. However, here the parental females carry free-*X*'s but have more heterochromatin than did the *X/X* mothers in the initial experiments (Tables 1 and 3); nevertheless, it is possible to compare the relative recovery of two identical progeny classes—the *X/X-Y* ♀ ♀ and *X/0* ♂ ♂. From *X/X* mothers, the *X0* male class is recovered only 0.09 as well as the female *X/X-Y* class, while from *X/X-Y* females, ♂ recovery is 0.37.

All the data, therefore, are consistent with the supposition that some product of sex chromosome heterochromatin, necessary for normal survival of the zygote, is packaged in the egg; *abo/abo* mothers produce eggs deficient or abnormal in respect of this product, but the abnormality can be partially compensated by additional sex chromosome heterochromatin either in the mother (e.g., extra synthesis of the product during the packaging) or in the zygote (e.g., extra synthesis at the time of utilization).

While it thus seems reasonable to ascribe differences in the magnitude of the *abo*-induced effect from different females to the sex chromosome heterochromatic content of the parental female, the quantitative problem of the magnitude of the effect as a function of the zygotic genotype remains: *XX* females survive better than *XY* males and there is no simple relationship between dosage of basal heterochromatin, either in the mother or in the zygote, and survival. In all probability this complex relationship is due to the nature of the measure used (i.e., relative survival) which must depend on an interaction among all developmental processes. A more comprehensible relationship is to be expected if one measures the product produced by the sex chromosome heterochromatin. One test, however, specifically indicating the lability of relative survival of the progeny of homozygous *abo* mothers has been carried out.

The standard experiment was performed at 19.5°C instead of the usual 25.5°C: i.e., stock *abo/In(2LR)Cy* males and females were crossed and *abo/abo* females (experimental) and *abo/In(2LR)Cy* females (control) were collected and crossed to $Y^{s}X{\cdot}Y^{L}$, *In(1)EN, y B/0* males and ♂ recovery calculated. Every three days a sample of cultures was shifted to 25.5°C. The results of this experiment, show-

ing also the approximate developmental stage at the time of the temperature shift, are presented in Figure 1.

It can be seen that while there is no temperature effect on the developing homozygous *abo* zygotes destined to be the parental females that will produce abnormal eggs (i.e., *abo* itself does not appear to be temperature sensitive), there is a profound temperature effect on the probability of survival of the developing zygotes exhibiting the effect.

SOMATIC EFFECTS OF *abo*

The experiments described until this point have all concerned the effect of *abo* during oogenesis. An experiment has been performed, however, which indicates that *abo* also acts in the somatic cells of both sexes. The experimental rationale was that if *abo* resulted in a deficiency of the functional products of sex chromosome heterochromatin during development similar to that in oogenesis, then a homozygous *abo* zygote developing from an abnormal egg (i.e., from a homozygous *abo* mother) should be less viable (owing to a lesser ability to compensate the *abo*-induced abnormality) than either an identical zygote from a heterozygous mother or a normal zygote from an *abo/abo* mother. The appropriate comparisons, along with the crosses generating the progeny, are shown in the upper part of Table 7; the lower part of the table gives the results of controls in which the same types of progeny appear as in the experimental set, but from the reciprocal crosses so that there is no maternal *abo* effect.

It can be seen that while there is only a very slight reduction in the viability of *abo* homozygotes when they are the progeny of heterozygous parents (compare

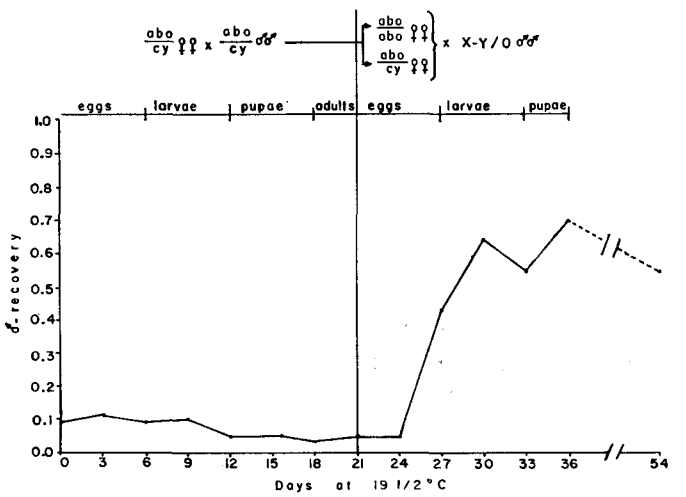

FIGURE 1.—The effect of development at 19.5°C of the *abo/abo* mothers (before day 21) and of her progeny (after day 21) on the *abo*-induced effect. After day 36, one set only was kept at 19.5°C for the entire experiment. Male recovery is calculated as in Table 3. The developmental stages shown refer to the progeny of the cross diagrammed above them.

TABLE 7

Results of crosses of females and males carrying normal, unmarked sex chromosomes, and with the indicated second chromosome constitution

Constitution of		Phenotype of progeny				
mother	father	+ ♀♀	*Cy* ♀♀	+ ♂♂	*Cy* ♂♂	Total progeny
abo/In(2LR)Cy	+/*In(2LR)Cy*	16 (16.7)	33 (33.3)	18 (16.7)	33 (33.3)	4021
abo/abo	+/*In(2LR)Cy*	28 (25.0)	26 (25.0)	20 (25.0)	26 (25.0)	2004
abo/In(2LR)Cy	*abo/In(2LR)Cy*	16 (16.7)	34 (33.3)	16 (16.7)	35 (33.3)	5178
abo/abo	*abo/In(2LR)Cy*	17 (25.0)	36 (25.0)	15 (25.0)	31 (25.0)	1881
+/*In(2LR)Cy*	*abo/In(2LR)Cy*	16 (16.7)	34 (33.3)	17 (16.7)	33 (33.3)	5645
+/*In(2LR)Cy*	*abo/abo*	24 (25.0)	26 (25.0)	25 (25.0)	26 (25.0)	6691
abo/In(2LR)Cy	*abo/In(2LR)Cy*	15 (16.7)	33 (33.3)	17 (16.7)	35 (33.3)	5520
abo/In(2LR)Cy	*abo/abo*	22 (25.0)	26 (25.0)	25 (25.0)	26 (25.0)	6418

The results are given in percentages; in parentheses are the Mendelian expectations.

lines 1 and 3 in both sets of crosses and lines 2 and 4 in the control set)—a reduction not necessarily due to *abo* itself, of course—homozygous progeny of homozygous mothers are recovered only about half as frequently (33 *abo*: 68 + compared to the expected 1:1; see line 4) as homozygous progeny of heterozygous mothers (compare lines 3 and 4 relative to 1 and 2 or lines 2 and 4) or heterozygous progeny of homozygous mothers (compare lines 2 and 4). The equivalent progeny of homozygous fathers (lower section of Table 7) show no gross departures from simple Mendelian expectations.

INTERPRETATION

The simplest interpretation of the foregoing data is that *abo*$^+$ is a regulator gene controlling the effective rate of synthesis of ribosomal RNA, the structural cistrons for which are in the sex chromosome heterochromatin. The mutant *abo* is, under this hypothesis, visualized as resulting in a reduction in ribosome production at all stages of the life cycle. Presumably this reduction is not very great since *abo* is not lethal, or even in most circumstances morphologically detectable. This latter point is obviously somewhat peculiar since a ribosome deficiency sufficient to cause a high probability of mortality would be expected to exhibit a bobbed phenotype. It would seem, therefore, that either *abo* is not involved in ribosome quantity as suggested here, or else that the relationship between ribosome quantity and the *bb* phenotype is not a simple one. Nevertheless, on this interpretation, mutant females would produce eggs with a deficiency of maternally-synthesized ribosomes which causes the observed zygotic mortality. The extent of the deficiency, and hence of the magnitude of the effect, is reduced if the mutant females have extra rRNA cistrons because their eggs would then have more ribosomes even though synthesis per cistron is reduced. The deficiency of maternal ribosomes can, evidently, also be partially made up by zygotic synthe-

sis, and thus the extent of the compensation is also related to the number of rRNA cistrons in the zygote.

Three other general considerations reinforce the suggestion that the *abo*-induced phenomena concern the quantity of ribosomes. First, phenotypic effects that depend on ribosomal quantity, unlike those resulting from enzyme deficiencies, would be expected to exhibit gene-dose dependence over a wide range of doses, as is observed with the compensation of *abo*-induced mortality. Secondly, it is striking that *abo* has its most profound effect during oogenesis when ribosome synthesis is generally very intense (BROWN and DAWID 1968). Finally, this interpretation suggests a very simple explanation for the temperature sensitivity noted earlier (Figure 1). It is possible, of course, that the temperature effect is directly the result of some temperature-sensitive component in the system. However, the only obvious effect of low temperature on Drosophila is that they develop slowly. Thus one can imagine that at the slower growth rate, the cells require fewer ribosomes and hence the developing progeny from ribosome-deficient eggs are less sensitive to the deficiency (i.e., have increased ♂ recovery) when they develop at the lower temperature. That this is a reasonable interpretation for a ribosome control system is indicated by the observation that in bacterial cells, the rRNA/DNA ratio increases dramatically with growth rate. This implies that rapidly growing bacterial cells require more ribosomes because there is a constant rate of protein synthesis per ribosome (MAALØE and KJELDGAARD 1966).

Thus it can be seen that all the data are satisfactorily accounted for by the hypothesis. Clearly, however, until biochemical studies are done (such experiments are currently being conducted by Mr. WILLIAM TAYLOR), other hypotheses can be entertained. These include control of other, yet to be identified, genes in the sex chromosome heterochromatin, or the production, by *abo*, of a defective component of ribosomes or a defective enzyme used in the synthesis of ribosomes or ribosomal RNA; however, direct control of ribosomal quantity seems the simplest working hypothesis. Under any hypothesis, *abo*$^+$ would be a component in the regulation of the amount, nature, or stability of some product synthesized by the sex chromosome heterochromatin.

It is a pleasure to express my appreciation to Mrs. A. ROSENFELD for making technically possible many of the experiments reported here.

SUMMARY

The genetic properties of a recessive autosomal point mutant found in a natural population of *Drosophila melanogaster* (*abo* = "abnormal oocyte") are described. It is suggested that *abo*$^+$ is a component, active throughout the life cycle, controlling the amount of cellular rRNA, the structural cistrons for which are located in the sex chromosome heterochromatin. The lines of evidence in favor of this interpretation are: (1) eggs produced by homozygous *abo* females are abnormal in respect of a maternally-produced constituent, as evidenced by a high probability of mortality, relative to heterozygous controls, among the developing zygotes;

(2) the abnormality in the egg can be compensated for by some postfertilization product of the sex chromosome heterochromatin, as evidenced by a decrease in the effect of homozygous *abo* in the parental female owing to increased sex chromosome heterochromatin contributed to the zygote by the parental (abo^+/abo^+) male; (3) similarly, the effect of homozygous *abo* in the mother is decreased by additional sex chromosome heterochromatin carried by the mother; (4) the class of *bb* mutants, deficiencies for rRNA cistrons, interact with the *abo*-induced effect such that heterozygous *bb* progeny of homozygous *abo* mothers are less viable than bb^+/bb^+ progeny of the same mothers, while heterozygotes for a large heterochromatic deficiency are even less viable; (5) *abo* has an effect, similar to that in oogenesis, on the somatic cells of both sexes as shown by a 50 percent reduction in the viability of *abo/abo* offspring of homozygous *abo* mothers compared with either identical zygotes from heterozygous *abo* mothers or *abo*/+ progeny of homozygous *abo* mothers; (6) ribosomal involvement is suggested by the fact that the *abo*-induced effect (a) is greatest at oogenesis, (b) shows gene-dose dependence over a wide range, and (c) exhibits a temperature sensitivity explicable in terms of known properties of ribosomes.—Additional genetic properties of *abo* and tests of some alternative explanations of the *abo*-effect are also presented and discussed.

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