THE INFLUENCE OF MINUTES UPON SOMATIC CROSSING OVER IN DROSOPHILA MELANOGASTER [1]

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THE Minutes are a group of dominant mutants, homozygous lethal, located on all four chromosomes of *Drosophila melanogaster*. Wherever located, they produce the same general physiological conditions resulting in reduced viability and lengthened larval life. STERN (1936) studied the mechanism of mosaic formation under the influence of certain Minutes and demonstrated that the mosaicism is a result of somatic crossing over. Apparently, the Minute physiology is able to influence chromosomal behavior resulting in an increased frequency of crossing over in somatic cells. Moreover, some interesting relationships between the locus of the Minute and the region of crossing over were shown to exist. While spots of sex-linked characters occurred with higher frequency when either sex-linked or autosomal Minutes were present than in non-Minute flies, sex-linked Minutes were more effective than autosomal ones. Third chromosome Minutes were capable of influencing crossing over in the third chromosome, while sex-linked Minutes were markedly less effective.

Furthermore, in the third chromosome studies an arm to arm correspondence was shown to exist: right arm Minutes more strongly influenced right arm crossing over than did left arm Minutes and the latter, in turn, were more effective in influencing the behavior of the left arm than were the right arm Minutes.

These findings have posed some interesting questions. Is the behavior of the third chromosome in the presence of a third chromosome Minute an isolated, specific phenomenon or is it a generalized expression of the Minute physiology? Do the relationships found to exist between the third and first chromosome apply also to the second and first chromosome? Accordingly, a series of experiments was undertaken to test the behavior of the first and second chromosomes under an experimental set-up similar to that of STERN's work.

THE EFFECT OF SECOND CHROMOSOME MINUTES UPON THE BEHAVIOR OF THE SECOND CHROMOSOME

Choice of materials

It was originally intended to use two left arm and two right arm Minutes for this work. However, $M(2)S^7$, a right arm Minute, was mistakenly used twice, once for itself and a second time in place of a selected left arm Minute

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with the result that the effects of two right arm and one left arm Minute comprise this study. The two MS^7 series are designated MS^7A and MS^7B. The other two Minutes chosen were $M(2)z$ on the left arm, and $M(2)l^2$ on the right. They are located as shown below. (Loci from BRIDGES and BREHME 1944.)

S	Mz	Bl	K	cn	MS^7	Ml^2	bw
1.3	12.9	54.8	55	57.5	77.5	101.2	104.5

Each of the three Minutes was combined in a chromosome with several mutant genes in such a way that crossing over would result in phenotypically detectable spots. Mutants affecting eye color and bristle structure were selected since they seemed to provide the most felicitous phenotypic effects and, at the same time, to utilize large surface areas. Star, Bristle, cinnabar and brown were chosen as markers. Their loci are indicated in the previously mentioned figure.

Before combining the Minute with the marker genes into the desired experimental and control genotypes, the second-chromosomal material of each Minute and marker stock was replaced on either side of the Minute or marker gene by Canton-S chromosomal material. For example, the chromosome to the right and to the left of the MS^7 locus was replaced, in a series of crosses, with Canton-S material. Similar replacements were made with each of the other Minutes. By this process, which may be referred to as cantonization, the second chromosomes of the experimental and control flies were made identical except for the presence, in the experimentals, of certain Minutes. It is to be noted, however, that there were small, specific regions on either side of each Minute and marker locus concerning which it was impossible to determine whether they had been replaced by Canton material.

Following is a diagrammatic representation of the three experimental chromosomes:

S	Mz	Bl	cn
1.3	12.9	54.8	57.5

S	Bl	cn	MS^7
			77.5

S	Bl	cn	Ml^2
			101.2

These chromosomes were maintained in stocks in which they were balanced over the $Cy\ al^2\ lt^3\ sp^2$ inversion complex.

For experimental purposes flies containing the above chromosomes were crossed to homozygous cinnabar brown flies thus deriving genotypes which may be represented as follows for the left and right arm Minutes respectively. Phenotypically, each fly was Minute, Bristle and cinnabar.

S	M	Bl	cn	+
+	+	+	cn	bw

S	Bl	cn	M	+
+	+	cn	+	bw

A stock, *S Bl cn/Cy,* was made from the cantonized *S, Bl,* and *cn* stocks, balanced over the Curly complex. These flies, in turn, were crossed to homozygous *cn bw* flies to derive the genotype *S Bl cn +/+ + cn bw,* which served as a control in this study.

It was found, as the work progressed, that the Star mutant used produced such phenotypically variable results that no attempt was made to identify non-Star tissue present within a genotypically Star eye. However, it was decided not to remove this mutant from the stocks but to leave it as a common element in the genotypic background.

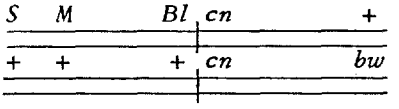
Crosses were made reciprocally and the flies were raised in half-pint bottles at 25°C on a standard agar, corn meal and molasses food mixture.

Analysis of results

STERN (1936) demonstrated that the presence of mosaics under his experimental conditions could be explained on the basis of crossovers involving two strands of a four-strand group. During the mitotic prophase of Diptera, homologous chromosomes are paired and each homologue " splits " into two sister chromatids (METZ 1916). The result is a tetrad-like structure as in meiosis. Indeed the resemblance that this cytological picture presents to meiosis is extraordinary, inasmuch as chiasmata are formed by the large autosomes of males and females and by the X's of females in neuroblast tissue (KAUFMAN 1934). However, in the mitotic anaphase the kinetochores divide and division is equational in relation to the kinetochores. Crossovers occurring between the kinetochore and a given locus heterozygous for a pair of alleles, followed by segregation, result in two cells homozygous for one or the other of the alleles. Tissues developing from such cells with newly acquired genotypes may be phenotypically different from tissues surrounding them and will, thus, be detectable. (It is to be noted that Star, Bristle and the Minutes are lethal when homozygous.)

The choice of the experimental genotypes stems from these considerations. Their significance will be discussed in the following paragraphs.

Mz. The accompanying diagram schematically represents the prophase chromosomes in the case of the left arm Minute.



The type of mosaic produced depends upon the region of crossing over. Exchanges along the right arm anywhere between the kinetochore and *bw* manifest themselves in the appearance of white patches in an otherwise cinnabar eye. Bristle mosaics cannot result from right arm crossovers. In the case of left arm crossovers, no eye mosaics may be produced. Two types of bristle mosaics, on the other hand, may be expected as a result of left arm crossovers. If the crossover lies in the region between the kinetochore and *Bl* then non-*Bl,*

non-*M* mosaics are the result. Crossing over between the *Bl* and Minute loci produces bristle mosaics that are *Bl*, non-*M*. Only the main bristles of the thorax and head were observed in the work concerned with the second chromosome.

It is reasonable to expect that double crossovers might have occurred. There are several instances where the phenotype of the mosaic is explained only by the occurrence of a double crossover. These cases are very rare, however, and are characterized by having one region in the right arm and the second in the left. It has, therefore, been assumed throughout that any given mosaic is the result of a single crossover except where single crossing over cannot account for the appearance of a specific mosaic. For example, wherever patches of white eye tissues have appeared in the *Mz* flies, it has been assumed that they are the result of a single crossover to the right of the kinetochore although the same result is possible following a specific three-strand double crossover. It may be added that in the case of the left arm Minute, a double

TABLE 1

Number and distribution of crossovers in the case of the left arm Minute.

Number of:	Mz			Control		
	Male	Female	Total	Male	Female	Total
Right arm c/o	1	2	3	0	2	2
Left arm c/o						
Between *Bl* & k	12	3	15	0	0	0
Between *M* & *Bl*	4	2	6			
Total c/o	17	7	24	0	2	2
		(0.49%)			(0.065%)	
Total flies inspected	2479	2397	4876	1431	1630	3061

crossover with one region between the kinetochore and *Bl* and the other between *Bl* and *M* would result in *M*, non-*Bl* spots. This type of mosaic was not found.

Had double crossovers involving the two arms of the second chromosome occurred, one might have expected instances where an eye mosaic was associated with a bristle mosaic. However, no such cases were found. (The bristle genotypes cannot be differentiated on the basis of the hairs of the ommatidia, so that identification of double crossing over involving an eye mosaic depends upon the appearance of a spot at the edge of the eye and overlapping the surrounding tissue.) The absence of such critical types of mosaics may serve as a measure of the very low frequency of double crossovers.

Table 1 summarizes the experimental data pertaining to the number and regions of somatic crossing over.

Among 3061 control flies, 2 occurrences of somatic crossing over were detected. The experimental data, except where otherwise indicated, were subjected to a statistical analysis according to a method of chi square described by FISHER and YATES (1949) and applying to tests of significance for 2×2 con-

tingency tables with one degree of freedom. This method is recommended where the smallest expectation of any class is less than 100. Whether the deviations from the control rate are significant at the 0.005 or 0.025 level of significance may then be determined by reference to table VIII of the above-mentioned publication. It is to be noted that for all the statistical analyses involving this method only one tail of the normal curve has been considered since deviation from the control series is in one direction only. However, if one wished to be especially conservative the levels may be doubled.

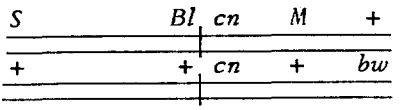
In the *Mz* series the difference in the proportion of mosaic flies as compared with the control series may not be attributed to chance as the deviations from the control rate are significant at the 0.005 level of significance.

The data provide no evidence in favor of the existence of any sex difference but, rather, a heterogeneity χ^2 test indicates that the distribution of crossovers between the two sexes is governed entirely by chance ($P > 0.05$).

Right arm crossovers have occurred in the presence of this left arm Minute. However, the magnitude is no greater than that of the control series and, therefore, no specific effect is likely. The probability that the observed deviation from an expected even distribution of crossovers between the right and left arm is due to chance is less than 0.005. The indications are, therefore, that *Mz* strongly favors crossing over in its own arm.

The data indicate, further, that a greater number of mosaics has resulted from crossing over between the kinetochore and the Bristle locus, a region 0.2 map units in length, than from crossing over between *Bl* and *M*, a region 41.9 units long. As there was no marker between *Bl* and the *M* locus it was not possible to determine how close to *Bl* the crossovers in the latter group occurred. However, these findings agree with STERN's for the third and X chromosome where it was shown that the crossovers induced by the presence of Minutes tend to occur close to the kinetochore.

MS[7] and *Ml*[2]. The following diagram schematically represents the prophase chromosomes of the experimental genotype in the case of the right arm Minutes.



Patches of white in a cinnabar eye may be expected as a result of crossing over along the right arm anywhere between the kinetochore and brown. The appearance of a *Bl*, non-*M* mosaic indicates a crossover between the kinetochore and the *M* locus. The presence of a Minute on the right arm may thus result in both eye and bristle mosaics when crossovers occur in that arm. Left arm crossovers are expressed phenotypically in non-*Bl*, *M* bristle mosaics, but their occurrence may not produce any eye mosaics. Table 2 presents the observed data.

A statistical analysis indicates that there is no evidence for the existence of a differential sex factor.

In testing the hypothesis that the controls and the experimentals are of the same population, and considering only one tail of the curve, it is found that for both MS^7 series the difference is significant at the 0.005 level ($P < 0.005$), whereas for Ml^2 the difference is significant at the 0.025 level ($0.005 < P < 0.025$).

It appears, thus, that the Minutes influence the behavior of the chromosomes in which they are located so that the frequency of somatic crossing over is increased above the control rate.

In the case of MS^7 and Ml^2 the region of crossing over could not be more precisely designated than as indicated in table 2. The presence of a white spot on a cinnabar eye indicates, merely, that crossing over had taken place somewhere between the kinetochore and the brown locus, as the phenotype of the

TABLE 2

Number and distribution of crossovers in the case of right arm Minutes. (Controls as in Table 1.)

Number of:	MS^7A			MS^7B			Ml^2		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Right arm c/o									
Between M & k	3	5	8	6	8	14	2	3	5
Between bw & k	6	5	11	4	7	11	3	3	6
Left arm c/o	1	1	2	2	0	2			0
Total c/o	10	10	21*	10	15	27**	5	6	11
		(0.34%)			(0.82%)			(0.26%)	
Total flies inspected	3076	3166	6192	1788	1486	3274	2044	2138	4182

*One double crossover scored twice; once among right arm crossovers, and again among left arm crossovers.

**Two double crossovers scored twice; once among right arm crossovers, and again among left arm crossovers.

eye mosaics produced as a result of crossing over between the kinetochore and *cn*, between *cn* and *M*, and between *M* and *bw* are indistinguishable.

On the other hand, a Minute bristle may be distinguished from a non-Minute bristle. Therefore, Bristle, non-Minute bristles may be detected, indicating that crossing over has occurred between the kinetochore and the Minute locus. Within the total of 14 eye mosaics in the MS^7 series, there were 3 which were associated with non-Minute bristles. These bristles immediately bordered the mosaic eye. Moreover, there were 5 mosaics in which only bristles were involved and these bristles were non-Minute. It was, therefore, possible to conclude that these eight mosaics developed from a genotype resulting from crossing over between the kinetochore and the Minute locus. The 11 cases of crossing over whose loci could not be more precisely defined than between the kinetochore and the brown locus were all eye mosaics.

Similar considerations have made it possible to ascribe 14 of the 27 right arm crossovers of the MS^7B series to the region between the kinetochore and the Minute locus.

In the Ml^2 series five of the eye mosaics were associated with non-Minute bristles permitting the crossover region to be limited to between the kinetochore and the Minute locus. The other six mosaics were eye mosaics only.

On the basis of what is known of the embryology of Drosophila it is possible to ascribe the presence of a combination eye color and bristle structure mosaic to a single crossover. BIRMINGHAM (1942) has shown experimentally that the eye discs give rise not only to ommatidia, but also to a predetermined area of the head hypodermis. However, it is only chance which will determine whether cells with the crossover genotype will form parts of the head as well as of eye tissue or whether they will give rise to eye tissue exclusively. The eye mosaics which are surrounded by bristles of the same phenotype as the rest of the fly offer no clue to a more precise determination of the crossover regions since Minute Bristle bristles may indicate that the crossover locus is between *M* and *bw*, or it may mean that the crossover locus is between the kinetochore and *M* but that none of the cells with the crossover genotype has taken part in the formation of the head hypodermis. On the basis of these considerations the relatively large number of mosaics indicating crossing over between the kinetochore and *M* may be taken as indication of the tendency of crossovers to occur toward the centromeric region.

The combined eye color and bristle structure mosaic is interesting for another, and perhaps more important, consideration. If, as it may be argued, the white facets of the mosaic are the result of a mutation either at the brown locus of the second chromosome or at the white locus of the first chromosome (significant in males only), then the presence of a non-Minute bristle adjoining this tissue would necessarily have to be explained by a second mutation at the Minute locus. Since the probability of such an occurrence is so extremely low it is much more reasonable to assume that crossing over has taken place between the kinetochore and the M locus. This has resulted in a rearrangement of the genetic factors to the right of the kinetochore but the *Bl* gene is still part of the resultant genotype, as it was originally.

In table 2 a double crossover has been scored for the MS^7A series, and two for the MS^7B series. Each of the three flies had a patch of tissue whose bristles were not-Minute and not-Bristle. Rather than evoke the highly improbable phenomenon of a double mutation, the situation is explained by a crossover to the left and to the right of the kinetochore. It is interesting to note that the left arm crossovers produced in the presence of the right arm Minutes were, in three of the four cases, associated with a right arm crossover. A similar phenomenon will be pointed out in the discussion on the behavior of the X chromosome.

The MS^7 and Ml^2 data were tested by means of the standard chi square test to determine whether each of these Minutes exerts a preferential effect upon the arm in which it is located. The hypothesis that the number of crossovers should be equally distributed between the two arms of the chromosome was tested and in all three instances the deviations from expectation were significant: $P < 0.01$. An arm-to-arm relationship is thus shown to exist.

The two MS^7 series will be regarded separately throughout this discussion.

Although their qualitative effects are similar, a marked influence upon the right arm of the second chromosome, the difference in the magnitude of effect between the two series is statistically significant so that the two sets of data may not be pooled ($P < 0.005$). Also, further quantitative differences will be pointed out subsequently. There is no apparent reason for the difference in the behavior of these two series of the same Minute. However, each was originally regarded as a separate entity and combined into the experimental stocks separately. Therefore, whatever differences exist in the genetic backgrounds of the two stocks may be responsible for the observed differences. This consideration leads to the conclusion that the Minute influence upon somatic crossing over is, itself, influenced by the genetic background.

Temperature experiments

In view of WHITTINGHILL's work (1937) on gonial crossing over it was decided to test the effect of heat, in the presence of Minutes, upon somatic crossing over. Both MS^1A and MS^1B, and Mz were used in this work. Eggs collected over 24 hours at 25°C were subjected to a temperature of 32°C for the next 48-hour period. At the end of that time, the larvae were returned to an environment of 25°C in which they completed their development. The exposure to heat was limited to a 48-hour period of the time during which the imaginal discs are rapidly growing by cell multiplication in order that the relatively brief exposure might cut down only slightly on the number of flies completing development in these cultures. A non-Minute series was carried along simultaneously. Table 3 summarizes the results obtained at 25° and at 32°C.

The heat treatment has increased the frequency of second chromosome somatic crossing over in the non-Minute as well as the three different Minute series. From a crossover frequency of 0.065% at 25° the non-Minute control frequency was increased to 0.32% at 32°. Within the Mz series the frequency was raised from 0.49% to 3.14%. An increase from 0.34% to 0.91% was brought about within the MS^1A series and the MS^1B series showed an increase from 0.82% to 4.64%.

For the three Minute series the differences in crossover frequency at the two temperature levels are significant at the 0.005 level of significance, whereas in the case of the non-Minute series the difference is significant at the 0.025 level of significance.

Moreover, none of the three Minute series may be considered samples of the same population as the control series at 32°, since the differences in frequency of crossing over are statistically significant at the 0.005 level of significance.

These results indicate that heat has an influence upon the processes involved in somatic crossing over and that this influence may be exerted in the absence of the Minutes.

Does the temperature shock increase the frequency of crossovers in the presence of the Minutes to the same degree as it does in their absence with the result that, in the Minute series, the number of crossovers is thereby equal

TABLE 3

Number and distribution of crossovers in Minute and non-Minute series at 25° and 32° C.

		Right arm between		Left arm between		Total crossovers	Total flies inspected
		k & M	k & bw	Bl & k	M & Bl		
Mz	25°		3	15	6	24 (0.49%)	4876
	32°		30	74	51	155 (3.14%) (76♂, 78♀)*	4935 (2119♂, 2816♀)
MS⁷A	25°	8	11	2		21 (0.34%)	6192
	32°	26	28	2		56 (0.91%) (20♂, 35♀)**	6136 (2780♂, 3356♀)
MS⁷B	25°	14	11	2		27 (0.82%)	3274
	32°	121	80	1		202 (4.64%) (91♂, 101♀)†	4355 (1956♂, 2399♀)
Control (non-M)	25°		2	0		2 (0.065%)	3061
	32°		9	1		10 (0.32%) (4♂, 6♀)	3108 (1345♂, 1763♀)

*1 fly with 2 mosaics.
**One double crossover.
†5 flies with 2 mosaics; 2 flies with 3 mosaics; 1 fly with double crossover.

to those brought about by the heat treatment plus those due to the presence of the Minute? Phrased differently, are the data compatible with an additive hypothesis or is the difference in crossover frequency at 25° and at 32° in the absence of Minutes significantly different from the difference in crossover frequency at 25° and 32° in the presence of Minutes? One might reasonably expect that some interaction has occurred between the Minute physiology and the conditions set up by the high temperature, producing some crossovers that would not have occurred in the presence of either single condition.

To answer this question, MR. H. M. HUGHES of the Department of Mathematics and the Statistical Laboratory of the University of California, Berkeley, devised a method involving the t test, in which it was assumed that the two differences are not significantly different from each other, but are considered as chance deviations in a universe of differences. The difference in percent crossing over between the rates at 25° and at 32° for the Minute and non-Minute series is determined. The difference between these two differences is then divided by the square root of the sum of the four standard errors of the difference, thus:

$$t = \frac{D}{\sqrt{\sum_{1}^{4} \frac{pq}{n}}}$$

With an infinite number of degrees of freedom the following statistical results have been determined:

$$\begin{aligned} Mz \quad & t = 8.28 \quad P < 0.001 \\ MS^1A \; & t = 1.76 \quad P = 0.084 \\ MS^1B \; & t = 10.17 \quad P < 0.001 \end{aligned}$$

For Mz and MS^1B the deviations based upon an additive hypothesis are significant, indicating that some sort of interaction has taken place between the conditions brought about by the heat shock treatment and the presence of the Minute, thus producing a multiplicative effect. In the case of MS^1A the lack of significance seems to support the additive hypothesis. However, on the basis of such an hypothesis a deviation of the magnitude observed between the two differences may be expected by chance once in only twelve trials.

Reference to table 5 will show that the same patterning of crossovers along the length of the second chromosomes obtains within the 32° series as was disclosed in the 25° series. For Mz there is still a preferential influence upon the left arm, although a relatively large number of right arm crossovers has been produced. The probability of obtaining as great a deviation from a random distribution between the two arms is less than 0.001. Moreover, the same distribution of crossovers within the left arm is present, the greater number occurring between the Bristle locus and the kinetochore. Statistical analysis indicates that the Mz influence upon the second chromosome is qualitatively the same at 25° and at 32°C; the probability that they are of the same population is greater than 0.05.

In both the MS^1A and MS^1B series the general patterning at 32° is the same as at 25°. There was no increase in left arm crossing over, but MS^1 seems to exert its effect almost exclusively upon the right arm despite the general increase in crossover frequency. Stated another way, the left arm remains refractory to right arm M influences.

One of the designated left-arm crossovers of MS^1A occurred together with a crossover in the right arm. This double crossover was noteworthy inasmuch as each eye contained a patch of white tissue on its dorsal margin; in addition, the right and left ocellar and right and left anterior and posterior vertical bristles were non-Bristle, non-Minute. Since the probability that this mosaic has resulted from two double crossovers in one individual is extremely low, it seems more reasonable that the mosaic patch has arisen from one original cell which received the crossover chromosomes. This view suggests that the crossover in question goes back to a cell present in tissue which later gave rise to the two optic discs. Although the early embryology of the optic discs in *D. melanogaster* has not been clearly determined a statement from POULSON (1950), suggesting that a posterior thickened region of the frontal sac showing signs of bilaterality early in embryological development gives rise to the antennal-optic discs, would indicate that such an event is possible.

Again, as within the 25° series there is a marked quantitative difference between the MS^1A and MS^1B sets of data. However, the influence of this

Minute upon the rate of somatic crossing over and the restriction of that influence to the arm of the second chromosome in which the Minute, itself, is located is consistent between the two groups.

THE INFLUENCE OF SECOND CHROMOSOME MINUTES UPON THE BEHAVIOR OF THE X CHROMOSOME

Choice of materials

The three second chromosome Minutes discussed in the previous section were tested to determine whether the X chromosome was influenced by their presence. Flies from the cantonized Minute stocks, balanced over Curly, were crossed to Canton flies in order to derive stocks with a Minute present in one of the second chromosomes and a Canton chromosome for the other. These flies were then crossed to homozygous yellow singed^3 flies. This permitted controls and experimentals to be derived from the same cultures. The experimental flies are Minute, the control flies have normal bristles:

$$\frac{+\quad +}{y\quad sn^3}\quad \frac{+}{M} \qquad\qquad \frac{+\quad +}{y\quad sn^3}\quad \frac{+}{Canton}$$

Females, alone, have been inspected in this cross as the males possess only one X chromosome. Yellow is located at 0.0, singed^3 at 21.0 and the kinetochore of the X chromosome is at 66.0.

Depending upon the region of crossing over three different types of sex-linked mosaics may be expected. (Any crossing over that has occurred on the part of the second chromosome has been disregarded.) A crossover between the kinetochore and singed^3, followed by equational segregation, will result in a *y sn*^{3} spot. Crossing over between singed^3 and yellow will produce a *y* mosaic, whereas the presence of a *sn*^{3} spot indicates the occurrence of a double crossover.

Analysis of results

Controls. Among 1427 control flies there were 48 instances of somatic crossing over on the part of the X chromosome. 43 of the mosaics were *y sn*^{3} in appearance, indicating that crossing over had occurred between the kinetochore and the *sn*^{3} locus. The other 5 mosaics were *sn*^{3}. These resulted from the occurrence of double crossing over.

It is difficult to explain the absence of yellow spots, the phenotypic manifestation of crossing over between *sn*^{3} and *y*. These experiments were done very early during the course of this study and yellow mosaics may have been overlooked. It seems reasonable that they should have occurred, especially since crossovers between *y* and *sn*^{3} have taken place at least 5 times as one of the regions concerned in double crossing over. However, table 9 suggests that crossovers in this region are very rare. In this connection it is interesting to compare the results obtained by AUERBACH (1945) in studying chromosome rearrangements in somatic cells following mustard gas treatment of *D. melanogaster* eggs. Evidence from this work indicates that somatic crossing over in the distal region of the X chromosome is very rare. Also where *y* and

TABLE 4

The effect of second chromosome Minutes upon the behavior of the first chromosome.

	Total flies inspected	Number of mosaics	% crossing over
Control	1475	48	3.25
Mz	642	94	14.64
MS^{7}A	221	45	20.36
MS^{7}B	461	61	13.24
Ml^2	623	64	10.27

sn spots were expected in equal frequency a preponderance of *sn* spots was detected. One explanation offered for this discrepancy was that yellow spots were missed with greater ease than *sn* spots. However, since *sn* spots could have resulted from double crossovers it is possible that the total of *sn* spots was augmented by the occurrence of double crossing over. This would indicate, as does the author's work, that it is possible that double crossovers have occurred involving the region between *sn^3* and *y* as one of the two loci, whereas crossing over between *sn^3* and *y* alone, was a much less frequent event. A similar occurrence has been pointed out previously. In all cases, but one, where the left arm of the second chromosome was influenced by the presence of a right arm Minute, the exchange occurred, along with crossing over in the right arm, as one of the regions involved in a double crossover.

All mosaics were limited to the abdominal region and all were small in area, none comprising more than 5 bristles. This condition indicates that the crossovers took place rather late in development. The frequency of X-chromosomal crossing over appears to be quite high as compared with the control rate detected for the second chromosome.

Minute series. Table 4 presents the data for the control and Minute series. A definite influence by second chromosome Minutes upon the X chromosomes is indicated.

The distribution and kinds of mosaics in these four experimental series were much the same as those found in the controls. Tables 5 and 6 summarize these data.

TABLE 5

X chromosome influenced by second chromosome Minutes: Distribution of single crossovers.

	Crossover between kinetochore and *sn*			Crossover between *sn* and *y*		
	Location of mosaic					
	Abdomen	Thorax	Head	Abdomen	Thorax	Head
Mz	83	1	0	0	0	0
MS^{7}A	34	3	0	1	0	0
MS^{7}B	44	0	1	0	0	0
Ml^2	54	1	0	0	0	0
Control	43	0	0	0	0	0

TABLE 6

X chromosome influenced by second chromosome Minutes: Distribution of double crossovers.

Location of mosaic	Abdomen	Thorax	Head
Mz	7	3	0
MS^1A	6	1	0
MS^1B	15	1	0
Ml^2	8	1	0
Control	5	0	0

Although the three Minutes exert the same general effect upon the X chromosome in relation to somatic crossing over there are some apparent differences in the magnitude of that effect. Testing these data for homogeneity, a χ^2 value of 15.16 is obtained. This value, with three degrees of freedom, is highly significant, indicating that the four Minute series may not be regarded as samples of the same population. However, since MS^1A and MS^1B are significantly different from each other ($\chi^2 = 5.2$, $0.025 > P > 0.005$) it cannot be stated whether these variations are definitely attributable to the differences in the Minutes, themselves, or whether they have been brought about by differences in the general genetic background.

Nevertheless, it is interesting to note that of all four Minutes used in this study, Ml^2 has had, consistently, the least effect upon the behavior of the second and the first chromosome. BREHME (1941) points out that of the Minutes that she studied Ml^2 had the least effect upon growth and development; the delay in puparium formation, as compared to the wild type, being only 13 hours. *Mz,* on the other hand, produces a two days' delay in puparium formation (BRIDGES and BREHME 1944). There appears to be, then, an indication of a parallelism between the " Minute effect " and the ability of any given Minute to induce somatic crossovers.

THE INFLUENCE OF A FIRST CHROMOSOME MINUTE UPON THE BEHAVIOR OF THE FIRST CHROMOSOME

Choice of materials

M(1)o, located at 56.6, was chosen as the sex-linked Minute for this aspect of the work. *M(1)o* females were crossed to yellow singed[3] males yielding the following experimental genotype:

$$\frac{+ \quad + \quad Mo}{y \quad sn^3 \quad +}$$

The control flies described in the previous section served as controls in this section of the work as well.

As previously, the crossover region may be identified by the appearance ot the resultant mosaic. A crossover between the kinetochore and the Minute locus will produce a spot in which the bristles are yellow and singed[3] in appearance. This may be distinguished from a mosaic resulting from a crossover

between the Minute locus and singed[3] since, in this latter instance; the bristles will be yellow, singed[3] and Minute. Similar considerations permit the identification of the crossover regions as summarized in table 7.

Results

A total of 52 mosaics, in 381 flies examined, was produced, as compared with 48 mosaics in a total of 1475 controls. *M(1)o* significantly increases the rate of somatic crossing over of the X chromosome above the control rate, the difference in frequency being significant at the 0.005 level.

As in the control series and that in which the effect of second chromosome Minutes upon the first chromosome was tested, the greatest number of crossovers took place to the right of the singed locus. In this series, however, it was possible to narrow down the region 35.6 units, by the presence of *M(1)o*, to within 9.4 map units to the left of the kineochore. Thus, in this instance, most

TABLE 7

X chromosome influenced by sex-linked Minute: Distribution of crossovers and location of mosaics.

	Distribution of crossovers					
	Single crossover between			Double crossover between		
	kinet & *M*	*M* & *sn*	*sn* & *y*	kinet & *M* *M* & *sn*	kinet & *M* *sn* & *y*	*M* & *sn* *sn* & *y*
M(1)o	41	6	2	0	3	0
Control	43 between kinet and *sn*			5 between kinet and *sn*, *sn* and *y*		

	Location of mosaics			
	Head	Thorax	Abdomen	Legs
M(1)o	8	25	17	2
Control	0	0	48	0

of the crossing over has occurred within 9.4 map units from the kinetochore. For the other X chromosome series there is no direct evidence to indicate that the greater part of the crossovers has taken place close to the fiber attachment point. However, STERN (1936) found this to be so in his work, and since the present work indicates that most of the crossovers have taken place to the right of the rightmost marker, it is reasonable to suggest the same situation holds true here.

In the control series all the mosaics occurred on the abdomen. The crossing over induced by the second chromosome Minutes also took place, for the most part, in cells concerned with the abdominal cuticle. However, with *M(1)o* almost 50% of the mosaics were located on the thorax, and 33⅓% of the remaining mosaics were present on the head region. Similar specifically localized regional differences were described by STERN (1936). Since the imaginal hypoderm of the thorax has a different origin from that of the abdomen this difference in the distribution of mosaics may reflect a differential sensitivity

of the cells of these anlagen to the presence of sex-linked Minutes as compared with the presence of autosomal Minutes.

STERN (1936) found that the sex-linked Minutes had a greater influence upon the behavior of the X chromosome than the third chromosome Minutes he worked with. This appears not to be so in the case of the second chromosome Minutes. The percent mosaics in the presence of *M(1)o* was 13.6%; for *M(2)S⁷A* the frequency was 20.6%. Statistically, this difference is significant at the 0.025 level of significance, indicating that the magnitude of effect of *M(2)S⁷* may be significantly greater than that of *M(1)o*.

The results of the other three autosomal Minutes were closer in magnitude to the *M(1)o* results than were those of *M(2)S⁷A*. *M(2)z* produced 14.6%; *M(2)S⁷B* 13.2%; and *M(2)l²* 10.2%. It would appear, therefore, that the autosomal Minutes used here were, at least, as effective as the sex-linked Minute.

Temperature experiment

STERN and RENTSCHLER (1936) found that, as regards the X chromosome, the frequency of somatic crossing over was lower at 30°C than at 25°C. Consequently, in view of the second chromosome findings, a series of crosses was set up to test the effect of the heat shock treatment upon the X chromosome. *M(1)o* females were crossed to *y sn³* males and the subsequent experimental treatment was the same as described for the series of second chromosome Minutes subjected to the 32° treatment. A Minute and non-Minute control series were, thus, treated simultaneously. Table 8 presents the results.

TABLE 8

A comparison of sex chromosome crossover frequency at 25° and at 32° C.

	Number of crossovers	Total flies inspected
M(1)o 25°	52 (13.64%)	381
M(1)o 32°	25 (4.70%)	532
non-Minute 25°	48 (3.25%)	1475
non-Minute 32°	18 (3.22%)	558

A statistical treatment discloses that the heat treatment with respect to the frequency of somatic crossing over is without significant effect upon the non-Minute flies (P = 0.9). Likewise, the difference in crossover frequency between the non-Minute series at 32° and the Minute series at 32° is without significance (P = 0.28). However, significance at the 0.005 level is attained when the Minute series at 25° is tested against the Minute series at 32°. Since the frequency is higher in the former instance, these analyses indicate that not only is the heat treatment without effect upon the X chromosome, but when heat is applied in the presence of a sex-linked Minute an interaction takes place which cuts out some crossovers that would have occurred in the presence of the Minute alone.

THE INFLUENCE OF FIRST CHROMOSOME MINUTES UPON THE BEHAVIOR OF THE SECOND CHROMOSOME

Choice of materials

M(1)o was used as the sex-linked Minute. A stock containing this Minute in the X chromosome and homozygous for *cn bw* in the second chromosome was established. Females of this stock were crossed to *S Bl cn* males to obtain the desired experimental genotype:

$$\frac{M(1)o}{+} \qquad \frac{S\ Bl\ cn\ +}{+\ +\ cn\ bw}$$

Results

Among a total of 3179 flies inspected there were 23 cases of somatic crossing over. The control frequency, as reported previously, was 2 in 3061 flies. The difference between these two frequencies is statistically significant at the 0.005 level.

All 23 crossovers involved the right arm of the second chromosome. The left arm, it seems, is refractory to most influences other than the presence of left arm Minutes.

ANALYSIS OF DATA OBTAINED FROM RECIPROCAL CROSSES

Data from the *M(2)z* crosses at 25° and *M(2)S^{7}A* at 32° were analyzed to determine if differences produced as a result of setting up crosses reciprocally were statistically significant. Table 9 summarizes the results of this analysis.

TABLE 9

Data derived from reciprocal crosses.

	Total	*cn bw* × *S M Bl cn*		*S M Bl cn* × *cn bw*		Statistical data
		No. of flies	No. of crossovers	No. of flies	No. of crossovers	
Mz	4876	3777	20	1099	4	$\chi^2 = 0.20$, P = 0.65
MS^{7}A	6136	4239	38	1897	18	$\chi^2 = 0.003$, P = 0.80

These data clearly indicate that the number of crossovers has been nonpreferentially distributed among the offspring of both types of crosses.

DISCUSSION

The Minutes provide rich opportunity for genetic and developmental studies. Their widespread distribution among all the four chromosomes of *Drosophila melanogaster* plus the similarity of their expression at whatever locus have made them especially interesting. The evidence presented herein indicates that the ability to induce somatic crossing over is an important aspect of the Minute

physiology. The induction of crossing over in somatic cells is not a property restricted to sex-linked Minutes or those located on the third chromosome, but three Minutes selected, with no previous bias, from the store of second chromosome Minutes have demonstrated this to be a general characteristic of the class of mutants to which they belong. Furthermore, it seems reasonable to suggest that the increased frequency of somatic crossing over depends as much upon the pairing condition of the Dipteran chromosomes as it does upon the Minute physiology. The significance of pairing during the mitotic division stages of Dipteran chromosomes is unknown as are the physiological conditions underlying the phenomenon, but it seems unlikely that crossing over between homologous chromosomes would occur under the Minute influence without the previously existing synapsis-like condition.

The arm to arm correspondence brought to light in the third chromosome study by Stern is quite clearly shown to exist by this work on the second chromosome. This ability of a genetic factor to control the behavior of the chromosome in which it is located has been met before. In maize studies of the *Ds* and *Ac* loci indicate that a heritable component may control a chromosome-break-inducing mechanism (McCLINTOCK 1951); and CROUSE (1943) discovered evidence in Sciara of an element of the X chromosome, at or near the kinetochore, which controls differential behavior.

The existence of the arm to arm relationship so precisely shown by *Mz, MS*^{7} and *Ml*^{2} gave the clue to the possible mistaken identity of the Minute in the *MS*^{7}*B* series. The types of mosaics in this series, interpreted on the basis of a left arm Minute, would necessarily be explained by crossing over in the left arm and right arm nonpreferentially. Since this appeared to be a marked departure from the behavior in the case of the other three Minutes, it was suggested by DR. E. NOVITSKI, that the Minute in question may not have been present on the left arm. Subsequent testing identified it as *MS*^{7}.

The work discussed in this paper points up the fact that subtle physiological differences exist within the group of the Minutes. Generally, the Minutes are able to increase the rate of somatic crossing over but certain qualitative and quantitative differences have been indicated during the course of this report.

In STERN's (1936) analysis of somatic crossing over it was shown that the greatest frequency of crossovers occurred adjacent to the spindle fiber attachment region for both the X and the third chromosomes. The data for the X chromosome and for the *M(2)z* series are consistent with these findings. The right arm of the second chromosome was not marked to permit a more precise delimitation of the crossover region than has been indicated. However, most of the crossovers have occurred at least between the kinetochore and the Minute locus. Furthermore, it has been shown by MATHER (1939) that the heterochromatic region of the X chromosome adjacent to the kinetochore is sensitive to high temperatures, as a result of which meiotic crossing over in this region is increased. It therefore seems likely that the Minutes are able to influence the heterochromatic regions of the mitotic chromosomes in such a way that crossing over takes place at a higher rate than occurs spontaneously. Moreover, it would seem that the Minute influence is greater upon the hetero-

chromatin located on the same side of the kinetochore as the Minute itself, and only occasionally does the Minute influence override the inhibitory effect of the kinetochore upon the heterochromatin on its other side, although the influence may extend to different chromosomes. Likewise, the heterochromatin of the X seems to be more responsive to the Minute influence than is heterochromatin located in the autosomes. However, this may, in fact, represent the existence of a greater inhibitory influence upon crossing over on the part of the autosomal kinetochore. The range of effect of the second chromosome kinetochore extends to the left and to the right of the kinetochore locus whereas in the case of the X chromosome the inhibitory influence extends in one direction only since the kinetochore is located in the heterochromatic substance very close to the end of the chromosome.

In the control series at 25° and at 32° and in the series testing the X chromosome influence upon the second chromosome, all the mosaics, with one exception, resulted from right arm crossovers. Furthermore, the effect of right arm Minutes has been limited almost exclusively to the right arm, while the influence of the left arm Minute has not been so markedly limited to its respective arm. There are several possible explanations that may be offered to account for this seeming bias in the direction of the right arm and although one or two seem more plausible than others none is presented as the one correct explanation.

1. Bristle mosaics were less readily detected than eye mosaics so that the phenotypic manifestations of left arm crossing over, which gave rise to bristle mosaics only, were frequently missed.

2. The two compound eyes of Drosophila offer a greater likelihood of detecting mosaics than the area of the thorax and head owing to developmental differences that exist between them.

3. The anlagen of the cells that give rise to bristles are less responsive to the influence of agents inducing somatic crossing over than are the anlagen of the eyes.

4. In the case of the right arm, the chromosome distance within which crossing over might occur and be detected phenotypically, was greater than in the case of the left arm.

5. The situation is a reflection of a basic difference in behavior between the left and right arms of the second chromosome.

In order to examine the first possibility the number and kinds of mosaics found within the series indicated were tabulated in table 10 and the relative frequencies of eye and bristle mosaics were noted to determine whether any discrepancy exists between the number of bristle mosaics to be expected and that which was actually found.

It is not meaningful to compare the total number of eye mosaics to the total number of bristle mosaics as some types of bristle mosaics are the result of double crossing over and, as such, are expected to occur infrequently. What is more significant is a comparison of the relative frequencies of eye mosaics and bristle mosaics that are the manifestations of one particular type of crossing over. For example, in the case of the right arm Minute, crossing over between

TABLE 10

Number and types of observed mosaics.

	Eye	Eye and bristle	*Bl*, non-*M*	non-*Bl*, *M*	non-*Bl*, non-*M*	Total
Total	180	24	212	2	95	513
Mz 25°	3	0*	6	0†	15	24
Mz 32°	30	0*	51	0†	74	155
MS^7A 25°	11	3	4	1	1*	20
MS^7A 32°	28	8	18	1	1*	56
MS^7B 25°	11	0	12	0	2*	25
MS^7B 32°	80	8	113	0	1*	202
Ml^2 25°	6	5	0	0	0*	11
ctl 25°	2	0*	0**	0**	0	2
ctl 32°	9	0*	0**	0**	1	10

*Result of double crossover involving two arms.

**Not expected.

†Result of double crossover involving one arm.

Note: Figures in total columns are for mosaics, not crossovers and may, therefore, not agree with totals in preceding tables.

the kinetochore and *M* will result in a patch of white eye tissue if the crossover cells are concerned with formation of the eye. However, if the cells contribute to formation of the imaginal hypodermis, Bristle, non-Minute bristle mosaics arise. Table 10 indicates that from the MS^7 series and the Ml^2 series 136 eye mosaics and 143 bristle mosaics, of the type described, were detected. The meaning of these frequencies becomes apparent if one considers the relative likelihood of discovering eye and bristle spots. It was possible to observe a difference in eye color involving, in one case, only one ommatidium. STEINBERG (1943) has shown that each ommatidium arises from a cluster of four cells observable in optic discs as early as at 72 hours of larval life. Therefore, there were available, between the two eyes, at least 1400 such units of cells from which phenotypic manifestations of crossing over might be derived. On the other hand, for the work concerned with the second chromosome, observations were restricted to the main bristles of the head and thorax. Thus, despite the presence of large surface area capable of providing evidence of crossing over, that evidence would be detectable only if the cells specifically giving rise to bristles contained the crossover genotype. The area to which observations were restricted provided 46 bristles developing each from two cells. Thus there is a smaller number of cells capable of expressing the crossover bristle genotype than in the case of eye pigmentation. On the basis of these considerations the reported number of bristle mosaics is remarkably high and the crossover frequency induced by the presence of the left arm Minutes becomes even more significant. Is the frequency of left arm crossing over even higher than the number of mosaics indicates or does it mean that the left arm Minutes, more frequently than other conditions, influenced crossing over so early in development that the resulting mosaic areas were large enough to include one or more bristles?

Referring once more to suggestion 1, in the left arm series, *Mz*, at 25° only

3 out of 24 mosaics were eye mosaics and at 32° 30 out of 155 involved eye facets. And, in addition, in the *Mz* series at both temperatures 89 non-Bristle, non-Minute spots were found. Since this is the type of mosaic expected in the control series from crossing over in the left arm (in the region between the kinetochore and Bl), it does not seem likely that it would be missed in one case if it were detected in another. On the basis of these considerations it is not likely that the first possibility offers a reasonable explanation.

The X chromosome work indicated the possibility that a group of cells giving rise to one type of imaginal structure might have a differential sensitivity to a particular Minute influence as compared with a group of cells giving rise to another type. Thus, it may be that the forerunners of bristle cells respond differently to the presence of Minutes than do those of ommatidial cells. However, it is only in the presence of non-left arm Minutes that no or few bristle mosaics were found. In the presence of left arm Minutes crossing over in cells which give rise to bristles occurred in higher frequency than was detected for the right arm in the presence of right, left or sex-linked Minutes. Therefore, it does not appear that bristle anlagen are insensitive to Minute influences.

The control, sex-linked Minute and right arm Minute genotypes are such that crossing over between the kinetochore and the Bristle locus, a distance of 0.2 map units, is the only left arm crossover that may be detected phenotypically since Star was not usable as an indicator. On the other hand, crossing over within the entire distance between the kinetochore and *bw,* 49.5 crossover units, in the case of the right arm was phenotypically detectable. This may account, at least partly, for the absence of left arm crossovers in all series except those in which left arm Minutes were tested.

It is to be noted, however, that in the two *Mz* series 61 percent of all left arm crossovers have taken place between *Bl* and the kinetochore. Furthermore, as already pointed out, somatic crossovers tend to occur in the region of the kinetochore. Therefore, it would seem that had crossing over taken place in those series not involving left arm Minutes, an appreciable number of them would have occurred between the kinetochore and *Bl* and would, thus, have been detectable.

On the basis of these latter considerations, it is reasonable to suggest that the left arm is generally less responsive to factors inducing somatic crossing over than is the right arm. This is reflected in the appearance of only one case of spontaneous crossing over (control at 32°C) and the lower frequency of Minute-induced crossovers except when the Minute is located in the left arm itself.

SUMMARY

1. Each of three second chromosome Minutes tested was able to increase the frequency of second chromosome crossing over in somatic cells of *Drosophila melanogaster*. One Minute, $M(2)z$, is located on the left arm, the other two $M(2)S^7$ and $M(2)l^2$, are located on the right arm.

2. The right and left arms of the second chromosome were differentially

influenced. The arm in which the Minute itself was located was more strongly influenced.

3. A temperature of 32°C, applied for 48 hours to larvae which ranged in age from 0 to 24 hours beyond hatching, resulted in a significant increase, above comparable series at 25°, in second chromosome crossing over in both Minute and non-Minute control flies. The presence of the Minute and the heat treatment interacted to produce a multiplicative effect resulting in some crossovers that would not have taken place in the presence of either condition alone.

4. The three second chromosome Minutes were shown to be able to induce crossing over of the X chromosome in somatic cells.

5. *M(1)o,* a sex-linked Minute, significantly increased the rate of somatic crossing over of the X chromosome at 25°C.

6. A 32° temperature shock treatment resulted in no increase, above the 25° series, on the part of the X chromosome both for Minute and non-Minute flies. Indeed, in the presence of the Minute and the heat treatment, some crossovers were eliminated that would have occurred in the presence of the Minute alone.

7. *M(1)o,* a sex-linked Minute, at 25°, significantly increased the frequency of crossing over of the second chromosome. Crossover loci were limited entirely to the right arm.

8. Very few mosaics produced as a result of left arm crossovers were detected except where the Minute used was located within the left arm. The right arm, however, was influenced by the presence of left arm and sex-linked Minutes. Several explanations have been offered to explain the greater responsiveness of the right arm.

9. The data indicate that crossing over takes place, for the most part, at the region of the kinetochore.

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