A CYTOGENETIC STUDY OF CROSSING OVER IN INVERSION HETEROZYGOTES OF *DROSOPHILA MELANOGASTER*¹

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THE recombinant offspring of *Drosophila melanogaster* females having free X chromosomes heterozygous for a long inversion are limited to even multiple crossover types. But from inversion heterozygotes in which the two X chromosomes are attached at their centromeric ends (= tandem metacentric compound X chromosome), single crossovers are frequently recovered. The synaptic configurations of these two structural heterozygotes are compared in Figure 1. Although this difference in recovery of single crossovers could be explained by supposing the products of single exchange in free X inversion heterozygotes to be inviable, there is no demonstrable lethality of zygotes produced by such females. The resolution of this apparent paradox was presented by STURTEVANT and BEADLE (1936) in their classic paper on the mechanics of crossing over in inversion heterozygotes; they proposed that single exchanges do occur in free X inversion heterozygotes, that the products of such exchange are invariably excluded from the functional egg nucleus, and that exclusion is accomplished by directed orientation of noncrossover chromatids towards the terminal products of the second meiotic division.

The cytological and genetic consequences of various kinds of exchange in free X inversion heterozygotes and in tandem metacentric compound X chromosomes

FIGURE 1.—Diagrammatic representations of the synaptic configurations of free X chromosomes heterozygous for a long inversion (A) and of the tandem metacentric chromosome (B). In the first meiotic division, two X centromeres will segregate in A whereas segregation of the Y and TM centromeres will occur in B.

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are summarized in Tables 1 and 2. The details of Table 1 have been supported by numerous cytological demonstrations of dicentric bridges and acentric fragments in a variety of plant and animal materials, but not in *Drosophila*; CARSON'S (1946) investigation of inversion heterozygotes of the dipteran *Sciara impatiens* most closely approximates the expectations for *Drosophila*, although it is curious that no bridge free first divisions were observed in some 300 eggs examined. These cytological observations have not been accompanied by genetic data except in the notable work on maize inversion heterozygotes where breakage products of first division bridges are not always excluded from the ovules (RHOADES and DEMPSEY 1953). The genetic analysis of free X inversion heterozygotes and of the tandem metacentric compound X chromosome of *Drosophila* has been extensively advanced and has required only refinements of the original STURTEVANT and BEADLE model (NOVITSKI 1955). However, genetic studies on other types of compound X chromosomes, especially the reversed acrocentric and reversed ring

TABLE 1

Cytological and genetic results expected from crossing over in free X inversion heterozygotes (after STURTEVANT and BEADLE 1936)

Rank	Tetrad	Type	Cytological results Divisions I and II*	Genetic results
E ₀		..	normal	NCO
E ₁		..	fragment + bridge	NCO
E ₂	{	2s	normal	½ NCO + ½ DCO
		3sa	fragment + bridge	½ NCO + ½ DCO
		3sb	fragment + bridge	½ NCO + ½ DCO
		4s	2 fragments + double bridge	Nullto X

* The cytological results listed here are produced at division I but may persist in the first division plane during division II.

TABLE 2

Cytological and genetic results expected from crossing over in tandem metacentric compound X females (after NOVITSKI 1951 and 1955)

Rank	Tetrad	Type	Division I	Cytological results Division II	Genetic results X-bearing gametes*
E ₀		..	normal	normal	TM
E ₁	{	a	fragment	fragment + bridge	lethal
		b	fragment	fragment	c X ^c + (1-c) TM
E ₂	{	2sa	normal	normal	½ TM + ½ CO TM
		2sb	normal	normal	TM
		3sa	fragment	fragment + bridge	lethal
		3sb	fragment	fragment	c X ^c + (1-c) CO TM
		4sa	2 fragments	2 fragments	X ^c
		4sb	2 fragments	2 fragments + double bridge	Nullto X

* TM=tandem metacentric, CO TM=tandem metacentric exhibiting homozygosis, X^c=single ring X chromosome, c=coefficient of non random disjunction.

(SANDLER 1958), have suggested a near or complete absence of single exchanges and a high frequency of zero and two exchange tetrads in these constructions. Consequently, the assumption that single exchanges occur freely within free X inversion heterozygotes is subject to question (SANDLER 1954).

In this report, cytological observations of bridges and fragments in the meiotic divisions of free X inversion heterozygotes and of tandem metacentric females of *Drosophila melanogaster* will be described, and the frequencies of these configurations will be compared with those expected from genetic data.

MATERIALS AND METHODS

The long inversion chosen for this study was *In(1)Y'* in which the inverted segment includes almost all the euchromatic portion of the X chromosome, or about 57 map units (for descriptions of this and other mutants, see BRIDGES and BREHME 1944). This inversion has been previously studied genetically (STURTEVANT and BEADLE 1936; NOVITSKI and SANDLER 1956) in both the free X and tandem metacentric combinations; in addition to these structural types, females homozygous for free X chromosomes of standard sequence were used in the present study. All females were heterozygous for various mutant markers and were three to five days old when producing eggs for either genetic or cytological data.

The eggs used for cytological preparations were obtained, one per female, by squeezing the egg from the uterus. Six to ten eggs were placed on an albuminized cover glass, covered with a drop of xylene for one minute to clear the chorion, punctured in the posterior region with a fine glass needle after removal of the xylene, and fixed by placing the cover glass in a Columbia dish containing Kahle's fixative. The fixed eggs were then prepared by the Feulgen whole mount procedure described by VON BORSTEL and LINDSLEY (1959). This method of egg collection provided a very poor yield of meiotic stages; a great majority of the eggs were in cleavage or later embryonic stages of development. In addition to the 100 eggs included in the data, there were 48 eggs in which no Feulgen positive material could be detected and 37 meiotic eggs rejected either because of being in stages inappropriate for classification for bridges and fragments or because of poor cytological quality. The male gamete was observed in transitional stages from elongated sperm head to rounded pronucleus in 90 of the 100 eggs recorded; no egg having more than one male gamete was seen. The eggs from the free X homozygotes and inversion heterozygotes were coded during cytological examination.

RESULTS AND DISCUSSION

The genetic results from all three types of females appear in Table 3. The data for the free X homozygotes and heterozygotes require little comment since they were quite typical of those ordinarily obtained from such crosses. In addition to the complete absence of single crossovers from the inversion heterozygotes, there was a reduced recovery of double crossovers. The frequency of double crossovers

TABLE 3

Genetic results of crosses of free X females homozygous for the standard sequence or heterozygous for In(1)y⁴ to sc males and of crosses of tandem metacentric females to sc cv v f males

Type offspring	Parental female constitution		
	$\gamma/\gamma^2\ cv\ v\ f$	$\gamma^4/\gamma^2\ cv\ v\ f$	$v\ \gamma^4\cdot f\ w^a\ \gamma/Y$
NCO	676 ♂ ♂	670 ♂ ♂	1799 TM ♀ ♀
SCO I	135 ♂ ♂	0	1528 X ^c ♀ ♀
SCO II	246 ♂ ♂	0	
SCO III	179 ♂ ♂	0	
DCO I, II	8 ♂ ♂	6 ♂ ♂	52 CO TM ♀ ♀
DCO I, III	39 ♂ ♂	12 ♂ ♂	
DCO II, III	42 ♂ ♂	13 ♂ ♂	
Patroclinous	1 ♂	37 ♂ ♂	6749 ♂ ♂
Others	1269 ♀ ♀	749 ♀ ♀	15*

* Superfemales, detachment males, etc.

plus patroclinous males (produced from nullo X eggs) permit an estimate of 14.7 percent double exchange tetrads (E₂) in these free X inversion heterozygotes; no estimates of E₁ and E₀ are possible from these data.

The results from tandem metacentric females do allow estimates of E₀, E₁ and E₂ tetrad frequencies, although such estimates may be subject to considerable error. This is because the single ring (X^c) chromosomes generated by exchanges in this compound are deficient for the tip of the chromosome and duplicated for its base; such rings are inviable in the male and as X^c/+ females are only 76.9 percent as viable as sib M-5/+ females (NOVITSKI and SANDLER 1956). Using this observation as a viability correction factor, the 1528 X^c females observed (Table 3) were corrected to 2022 for further computations. (The senior author's choice of *In(1)y⁴* for these experiments is most embarrassing because of the availability of a tandem metacentric chromosome especially constructed (NOVITSKI and LINDSLEY 1950) to avoid generated ring inviability). Using equations presented by NOVITSKI (1951), the following estimates were calculated from the tandem metacentric data: c = .70, E₀ = 13 percent, E₁ = 79 percent, and E₂ = eight percent.

It is perhaps gratuitous to describe meiosis in *Drosophila melanogaster* females as less than favorable cytological material; the figures are extremely small, the "large" chromosomes are seldom distinguishable, the "small" chromosome IV is seldom detectable, and the entire process subsequent to fertilization at Metaphase I is apparently rapid. The second meiotic division spindles are arranged in tandem near the anterodorsal surface of the egg, and the terminal product of meiosis nearest the center of the egg becomes the functional egg nucleus. Our limited observations of meiosis in standard sequence free X homozygotes were in accord with previous descriptions (SONNENBLICK 1950).

Reference to Table 1 shows that in free X inversion heterozygotes single and three strand double exchanges lead to the formation of a dicentric bridge and an

acentric fragment at the first meiotic division; these structures are illustrated in Figures 2A and 2B. In a few cases, the bridge extended from pole to pole, but in most cases the bridge was situated either midway between the poles or in one or the other half of the figure. In 16 of 17 division I figures having a bridge, a small acentric fragment was also observed, usually midway between the poles and near, but not in, the longitudinal axis. The coiled structure of the bridge and the remoteness of the fragment in Figure 2B were atypical. Some, if not all, of the bridges formed in the first division persisted during the second division (Figures 2C and 2D); in these cases, the bridges, and the fragments if present, were located

FIGURE 2.—Drawings (ca. 1975 $\times$) of meiotic figures from free X inversion heterozygotes (A-D) and from tandem metacentric females (E-H). A, B, E, and F are division I figures and C, D, G and H are division II figures. The figures drawn were selected as those most nearly parallel to the microscopic field, and in all cases, the lower end of the figure was directed towards the center of the egg.

in the plane of the first division spindle, indicating their exclusion from the egg nucleus.

Single and three strand double exchanges in the tandem metacentric chromosome cause fragment production in the first meiotic division (Table 2), but there is no accompanying bridge since the X and Y centromeres segregate in the first division; this cytological expectation is illustrated in Figures 2E and 2F. No cases of first division bridges were found in the tandem metacentric material. Among the single and double exchanges which cause fragment production in the first division, only half are expected to cause second division bridges, assuming no strand preference in exchange participation. Nine second division figures were observed with fragments in the first division plane, and among these, five exhibited bridges in one of the two second division planes (Figure 2G), two revealed no bridges in either second division plane (Figure 2H), and two others could not be classified for bridge presence or absence in one of their second division planes. It is intended that the fate of these second division bridges will be described in a separate report.

Four strand double exchanges are expected to generate two acentric fragments in the first division; these fragments should be accompanied by double bridges in division I of free X heterozygotes and in division II of tandem metacentrics. No configurations of this sort were observed, but this fact is not significant for if the frequency of E_2 tetrads is taken to be 14.7 percent and if one fourth of these are four strand doubles, then only three figures of this type were expected among the 85 figures observed.

The simplest approach to a quantitative treatment of the cytological results is to consider the frequencies of normal meiotic figures, that is, those figures which had neither bridges nor fragments. Since the frequency of normal figures equals $E_0 + \frac{1}{4} E_2$ in all cases (Tables 1 and 2), direct comparisons as well as sums can be made (Table 4). In both the free X inversion heterozygote and the tandem metacentric data, there were large differences in normal figure frequencies between the first and second divisions; however, these differences were not statistically significant and, being of opposite sign, cancelled each other when totalled for both types of chromosomes. Thus the data revealed no real differences in normal figure frequency between the first and second divisions or between the two kinds of chromosomes; furthermore, they indicate that $E_0 + \frac{1}{4} E_2 = 34$ percent. This estimate may be compared with that calculated from the tandem metacentric genetic results where $E_0 + \frac{1}{4} E_2 = 15$ percent.

We do not believe the difference between the cytological and genetic estimates of $E_0 + \frac{1}{4} E_2$ to be significant because of probable sources of error which may have caused either the cytological estimate to be too high, the genetic estimate to be too low, or both. The classification of a meiotic figure as normal depended upon failure to observe either an acentric fragment or a dicentric bridge. The fragments were so small as to be difficult to detect if removed from the vicinity of the meiotic figure; in the free X inversion heterozygote material, there were three cases in which a bridge, but not the expected fragment, was observed. This source of error

TABLE 4

Cytological results observed in meiotic figures in eggs from free X females homozygous for the standard (+) sequence or heterozygous for $In(1)y^4$ and from tandem metacentric (TM) females

Female constitution	Meiotic division	Number of eggs			
		Total	Normal*	With fragment	With bridge
+/+	I	8	8	0	0
	II	7	7	0	0
	I + II	15	15	0	0
$In/+$	I	21	4 (.19)	16	17
	II	9	5 (.56)	2	4
	I + II	30	9 (.30)	18	21
TM/Y	I	44	18 (.41)	26	0
	II	11	2 (.18)	9	5 (+2?)
	I + II	55	20 (.36)	35	..
$In/+$ and TM/Y	I	65	22 (.34)		
	II	20	7 (.35)		
	I + II	85	29 (.34)		

* Numbers in parentheses are frequencies of normal with respect to total eggs.

may have been prominent in the first division tandem metacentric material where detection of an exchange depended solely upon observing the acentric fragment. It is also possible that broken bridges, if sufficiently retracted towards the poles, would have escaped cytological detection. Earlier reference has been made to the dependence of the tetrad rank frequencies inferred from the genetic data upon a correction for X^c female inviability. Although there is no question but that such a correction is appropriate, the actual amount of the correction is necessarily arbitrary. If the number of X^c females had been adjusted by a factor of 1.5 rather than 1.323, then the estimates of E_0 , E_1 and E_2 would have been 21, 69 and ten percent, respectively, and the value of $E_0 + \frac{1}{4} E_2 = 23.5$ percent; however, the value of $c = 0.88$ on this basis is suspiciously high. In view of these considerations, it is satisfying that the genetic and cytological results agree as well as they do.

SUMMARY

Genetic and cytological data were collected from eggs produced by *Drosophila melanogaster* females of three structural types with regard to their X chromosomes, namely, free X standard sequence homozygotes, free X inversion heterozygotes and tandem metacentric compound X females; the inversion used was $In(1)y^4$ which involves almost all the euchromatin of the X chromosome and which composes one of the arms of the tandem metacentric chromosome.

The cytological observations were in qualitative agreement with those predicted by previous genetic analyses: bridge and fragment configurations were observed

in the first division plane of free X inversion heterozygotes, while in the tandem metacentric eggs, fragments appeared in the first division plane and bridges appeared only in the second division plane. Considered quantitatively, the cytological results from free X inversion heterozygotes and tandem metacentrics were equivalent. The incidence of meiotic figures having neither bridges nor fragments and representing $E_0 + \frac{1}{4} E_2$ tetrads was 34 percent; this value and that of 15 percent estimated from genetic data were judged to be compatible when probable sources of genetic and cytological error were considered.

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