THE TIME OF INITIATION OF SEGREGATIONAL PAIRING BETWEEN
NONHOMOLOGUES IN *DROSOPHILA MELANOGASTER*:
A REEXAMINATION OF w^{m4} *

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THE distributive pairing model of meiosis for the female of *Drosophila melanogaster* (R. F. GRELL 1962a) postulates a chronological series of events which includes exchange pairing, exchange, distributive pairing, and segregation, in the order given. The model was dictated by genetic data, derived from studies of nonhomologous pairing, which showed that the frequency with which nonhomologues paired, as measured by their segregation, did not influence the frequency with which homologues paired, as measured by exchange, even though the same chromosome was involved in both processes. Furthermore, nonhomologous pairing, although selectively involving noncrossover chromosomes, did not produce the noncrossovers that it utilized, since it did not increase their frequency. Thus, two independent pairings were indicated and a temporal order was conferred upon them such that the homologous type, as well as the process of exchange itself, necessarily preceded the nonhomologous type. The distributive model represents the single sequence consonant with this genetic information.

Subsequent studies, including those of secondary nondisjunction (R. F. GRELL 1962b), of heterozygously inverted heterologues (ROBERTS 1962), of compound chromosomes (E. H. GRELL 1963, 1970), of free fourth chromosomes (R. F. GRELL 1964b), of heterozygous translocations (CHANDLEY 1965; R. F. GRELL, unpublished), and particularly free X duplications (R. F. GRELL 1967) have extended and clarified certain aspects of the model; but, more importantly, they have in every case provided additional evidence as to its validity.

In 1967, MERRIAM reported certain data which he interpreted to mean that *nonhomologous associations, bearing segregational consequences, were established prior to exchange*. His conclusion was based upon a genetic test of a particular inverted X chromosome known as white-mottled 4. The test was intended to distinguish between the distributive model and an "alternative" model proposed by NOVITSKI (1964). In reality, the model which MERRIAM tested turned out to be not the NOVITSKI model but a third model which will be designated the MERRIAM model. A comparison of the three models can be found under RESULTS AND ANALYSIS.

MERRIAM's test depended first upon the observation that a Y chromosome, added to females carrying w^{m4} in homozygous condition, apparently decreased X

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exchange and increased noncrossover *X* tetrads, and secondly upon the demonstration that these alterations in *X* exchange by the *Y* chromosome (presumably marking the position of the *Y* at exchange) were eliminated through the introduction of a nonhomologue which competed successfully for pairing with the *Y*. Unfortunately, in all but one instance, MERRIAM's data were derived solely from an examination of the proximal two-thirds of the genetic length of the w^{m4} chromosome. Earlier studies (R. F. GRELL 1962b) of the effect of a *Y* chromosome on crossing over between two normal *X*'s had shown that while the *Y* significantly reduced proximal exchange, it also caused a more than compensatory increase in distal exchange so that the overall effect of the *Y* was a slight increase in total *X* exchange. At the same time, the frequency of noncrossover *X* tetrads was found to be unaltered by the *Y*. In view of this information, it seemed appropriate to re-examine the effect of a *Y* chromosome on crossing over and on tetrad frequencies in w^{m4} in both the absence and the presence of a successful nonhomologous competitor for the *Y*. Obviously this reexamination should include the distal third as well as the proximal two-thirds of the chromosome.

The present investigation has failed to confirm any of MERRIAM's findings. The w^{m4} chromosome, much like the normal *X*, displays both a proximal decrease and a compensatory distal increase in crossing over in the presence of the *Y*, so that total exchange remains unaltered. Tetrad analysis reveals that the frequency of noncrossover *X* tetrads is virtually identical whether the *Y* is present or absent. The introduction of a successful nonhomologous competitor for the *Y*, utilizing both the nonhomologue tested by MERRIAM as well as two alternative nonhomologues not previously tested, fails in each case to alter the depression in proximal *X* exchange induced by the *Y*. This is the result predicted by the distributive model, in which nonhomologous associations follow exchange, but is incompatible with the MERRIAM model, in which nonhomologous associations precede exchange.

Finally, calculations of the sum of the frequencies with which the *Y* segregates both from the *X* chromosomes and from the newly introduced nonhomologue indicate that the frequency of *Y* pairing, as computed from the MERRIAM model, would have to significantly exceed the maximal limit of 100% for each competitor tested; whereas the frequency of *Y* pairing, as calculated from the distributive model, either falls within or reaches this limit but in no case significantly exceeds it.

MATERIALS AND METHODS

The inverted *X* chromosome, white-mottled 4 ($In(1)w^{m4}$), which has been used in all of these studies, is represented diagrammatically in Figure 1. The inversion involves a distal break at 3C1-2 and a proximal break to the left of bobbed (*bb*, 66.0) in *hC* or *hD*, so that the locus of white (w^+ , 1.5) is now relocated next to proximal heterochromatin and, conversely, the distal portion of the proximal heterochromatin now occupies an extremely distal location. The rearrangement of w^+ results in a variegated position-effect for this locus that is phenotypically recognizable in the hemizygous or homozygous condition as a mottled eye of near-white background with numerous small reddish dots. The addition of a *Y* chromosome to a female carrying the inversion homozygously or the addition of a second *Y* to a male carrying the inversion

NORMAL X

$ln(1)w^{m4}$

FIGURE 1.—Diagram of normal X chromosome and $ln(1)w^{m4}$, with arrows on normal X marking break points for induction of $ln(1)w^{m4}$. Heterozygous markers, used to measure crossing over in $ln(1)w^{m4}$, demarcate three chromosomal regions: distal, medial, and proximal. Interruption in bar above w^{m4} indicates distal region 1, which was included in present studies but not in earlier ones.

hemizygotously suppresses the variegation and restores the eye to approximately wild type. This property of the Y chromosome has been used to identify its absence or presence.

The markers that were introduced heterozygotously into the w^{m4} homozygote in order to study crossing over were yellow (y' , 1–0.0), crossveinless (cv , 1–13.7), wavy (wy , 1–41.9), and forked (f , 1–56.7). The new sequence of these markers in the inverted w^{m4} chromosome, progressing in a proximal direction from the tip, is y' , f , wy , and cv , so that the following three crossover regions are demarcated: (1) a distal region between y' and f , (2) a medial region

between *f* and *wy*, and (3) a proximal region between *wy* and *cv* (see Figure 1). The most proximal portion of the w^{m4} chromosome, between *cv* and *w*, has never been studied. A single attempt to introduce the marker echinus (*ec*, 1-5.5) into this region, using triploid females to increase crossing over, was unsuccessful. In its normal location the region measures about 11 crossover units, but its relocation proximally would be expected to drastically reduce exchange within it because of the centromere effect (BEADLE 1932). Studies of the most proximal portion of the normal *X* chromosome have repeatedly shown that practically all crossovers here originate as double or higher rank exchange tetrads (R. F. GRELL, unpublished). This means that inclusion of the region between *cv* and *w* would be expected to increase the genetic length of the w^{m4} chromosome by a few units and to transform some small portion of the single crossover class into doubles (or doubles into triples) but would not be expected to transform noncrossovers into crossovers. Transformation of singles into doubles has the effect of augmenting the noncrossover tetrads.

Disjunction of the *X* chromosomes was followed among the progeny by the presence of Bar (*B*, 1-57.0) in the attached-*XY* chromosome of the father. In this way the progeny resulting from regular disjunction of the maternal *X* chromosomes, which are *B* females and *B*⁺ males, are readily distinguishable from progeny resulting from nondisjunction of the maternal *X* chromosomes, which are *B*⁺ females and *B* males.

The first part of the study reexamined the effect of a *Y* chromosome on crossing over in females that were homozygous for the w^{m4} chromosome. Sisters, some of which lacked a Canton-S *Y* chromosome and some of which carried one, were employed for this purpose. The genotypes of the female and male parents used in this cross were:

$$In(1)w^{m4}, cv wy f / In(1)w^{m4}, \gamma^1(Y^{C-S}) \text{♀} \times \overline{XY}, \gamma B/Y \text{♂}$$

Total crossover values as well as regional crossing over for the three segments defined by the four markers were compared among male progeny from the *XX* and *XXY* sisters. The crossover data were then converted to tetrad frequencies (WEINSTEIN 1936) to determine the effect of the *Y* chromosome on the frequency of noncrossover *X* tetrads.

The second part of the study examined the effect upon crossing over in the *X* chromosome of a nonhomologue which competed successfully with the *X*'s for pairing with the *Y* chromosome. This series of experiments constituted still another method for testing whether nonhomologous pairing preceded or followed exchange, since only in the former case would *X* exchange values be expected to be altered by pairing between the *Y* and a nonhomologue. The three nonhomologue chromosomes that were employed for the tests included a multiply inverted second chromosome known as *SM1*, which carries the dominant marker Curly (*Cy*, 2-6.1); a free second chromosome duplication known as *Dp* (2;*f*)1, which carries the tip of 2*R* including the normal allele of speck (*sp*⁺, 2-107.0); and a free fourth chromosome which carries the dominant marker cubitus interruptus-Dominant (*ci*^D, 4-0). The genotypes of the females and males used for the three tests were:

$$\text{Cross \#1: } In(1)w^{m4}, cv wy f / In(1)w^{m4}, \gamma^1(Y^{C-S}); SM1, Cy/T(2;3)A, Bl \text{♀} \\ \times \overline{XY}, \gamma B/Y \text{♂}$$

$$\text{Cross \#2: } In(1)w^{m4}, \gamma^{2sc} cv wy f / In(1)w^{m4}, \gamma^1(B^SY); sp^2/sp^2/Dp(2;f)1, sp^+ \text{♀} \\ \times al^2 b c sp^2/al^2 b c sp^2 \text{♂}$$

$$\text{Cross \#3: } In(1)w^{m4}, cv wy f / In(1)w^{m4}, \gamma^1(Y^{C-S}); T(3;4)86D, bx^{34e}/T(3;4)86D, \\ bx^{34e}/ci^D \text{♀} \times \overline{XY}, \gamma B/Y \text{♂}$$

In Cross #1 the identification of the nonhomologue, *SM1*, among the progeny was accomplished by the dominant marker *Cy*, whereas the identification of the *Y*, as noted above, was achieved through its suppression of w^{m4} variegation. The frequency of segregation between the *Y* and *SM1* among the progeny was used to calculate the frequency of nonhomologous association between the *Y* and *SM1* by means of the expression $a \approx 1 - 2n$ where a = frequency of association and n = frequency of nondisjunction (GRELL and GRELL 1960). The introduction of translocation (2;3)*A* heterozygously into the females served as the device whereby associations of the *Y* were selectively restricted to only one of the noncrossover second chromosomes. The way this operates is that when *Y*-bearing females are heterozygotes for a multiply inverted second

chromosome, the *Y* associates nonpreferentially with the inverted 2 and the normal 2 so as to present among the progeny the appearance of independent assortment between the *Y* and the two second chromosomes. Progeny counts from single females, however, reveal an approximately 50% reduction when the *Y* is present (OKSALA 1958; R. F. GRELL, unpublished). This lethality is attributable to *Y*-induced nondisjunction of the second chromosomes, arising from nonhomologous associations between the *Y* and either second chromosome with random assortment of the other. It is possible, however, to limit *Y* associations to the inverted 2 by involving the homologue in a translocation with chromosome 3, e.g., $T(2;3)A$. In this case the translocated 2, although necessarily a noncrossover as frequently as the inverted 2, is, by virtue of the translocation, part of a third chromosome crossover bivalent and, as such, unavailable for nonhomologous associations. The situation would be equally effective if it were reversed so that the inverted 2 was part of the translocation and the normal 2 was free to pair with and segregate from a nonhomologue, (i.e., the arrangement of genetic material within the chromosome is irrelevant in nonhomologous pairing).

In Cross #2 identification of the nonhomologue among the progeny was accomplished by the presence of *sp*⁺ in the duplication in conjunction with the presence of a recessive allele of speck in all of the parental second chromosomes. Thus all progeny carrying the duplication were *sp*⁺ while those lacking it were *sp*². For this cross it was necessary to use the marked Bar of Stone *Y* (*B^SY*), since *Dp(2;f)1* itself causes suppression of *w^{m4}* variegation. Since the dominant mutant *B* had been preempted by the *B^SY*, *X* nondisjunction was followed by using *γ*¹ and *γ*² in the maternal *X*'s and *γ*⁺ in the male *X*, so that regular females and males were *γ*⁺ and *γ*¹ or *γ*², respectively, and exceptional females and males were *γ*² and *γ*⁺, respectively.

In Cross #3 the dominant marker *ci^D* in chromosome 4 provided the means of identifying the nonhomologue among the progeny, whereas identification of the *Y* was again accomplished by the suppression of the *w^{m4}*. This situation resembled Cross #1 in that it became necessary to introduce a rearrangement into the mothers, in this case a homozygous 3;4 translocation, to ensure that the free fourth chromosome marked with *ci^D* was usually the only 4 available for nonhomologous pairing with the *Y*. As a result of this arrangement the two unmarked fourth chromosomes were generally part of a third chromosome crossover bivalent and hence unavailable for association with the *Y*.

For fuller descriptions of the mutants and the rearrangements used, see LINDSLEY and GRELL (1968).

In crosses #2 and #3 the association frequency between the *Y* and the nonhomologue was calculated as in Cross #1, while in Crosses #1, #2, and #3, the crossover studies of *w^{m4}* and the tetrad analyses of the crossover data followed the procedures given under part one.

In all crosses single, newly eclosed virgin females were mated in vials to three males for 24 hr. The parents were then transferred to bottles and maintained at 25°C ± 0.5° until eclosion. Crossing over was studied only among the regular male progeny.

RESULTS AND ANALYSIS

The effect of an unmarked *Y* chromosome on both regional and total crossing over in homozygous *w^{m4}* from three identical but independent tests is given in Table 1. In each test the *Y* causes a proximal decrease in exchange (column 3), as well as an approximately equivalent distal increase in exchange (col. 1), so that total values are unaltered (col. 4). When the results from the three tests are combined (Table 1, lines 7 and 8), the effect of the *Y* is seen to be a significant decrease proximally, no significant alteration medially, and a significant increase distally. Total exchange values remain unaltered by the *Y* (i.e., $w^{m4}/w^{m4} = 52.7 \pm 0.6$; $w^{m4}/w^{m4}/Y = 52.5 \pm 0.7$). It should be noted that had the distal region been neglected, the appearance of a significant reduction in total exchange would have been found. Thus, by using the present data but omitting the distal region, total crossing over is apparently reduced by the *Y* from 35.0% in w^{m4}/w^{m4}

TABLE 1
Effect of the Y chromosome on crossing over, nondisjunction, and E_0 frequency in homozygous $ln(1)w^{m4}$

	Distal (region 1) $y-f$	Crossing over (%) [*] Medial (region 2) $f-uy$	Proximal (region 3) $uy-cv$	Total $y-cv$	Number	X Nondisjunction (%)	E_0 (%)
Test 1 { Y^{c-s}	20.7	14.8	18.2	53.7	1393	6.3	11.7
0	↑+2.9 17.8	13.0	↓-4.5 22.7	53.5	1964	0.2	12.1
Test 2 { Y^{c-s}	21.1	11.0	21.4	53.5	688	4.9	11.0
0	↑+5.4 15.7	13.3	↓-4.1 25.5	54.5	834	0.2	8.2
Test 3 { Y^{c-s}	22.2	13.3	16.4	51.9	3555	5.7	12.5
0	↑+4.2 18.0	14.8	↓-3.0 19.4	52.2	5203	0.1	13.2
Average { Y^{c-s}	21.7 ± 0.6	13.4 ± 0.5	17.4 ± 0.5	52.5 ± 0.7	5636	5.81	12.1
0	↑+4.0 17.7 ± 0.4	14.2 ± 0.4	↓-3.4 20.8 ± 0.5	52.7 ± 0.6	8001	0.15	12.4

* Number to right of arrow designates direction and amount of change induced by Y chromosome.

females to 30.8% in $w^{m4}/w^{m4}/Y$ females; alternatively, omission of the proximal region would lead to an apparent increase in crossing over by the Y from 31.9% in w^{m4}/w^{m4} females to 35.1% in $w^{m4}/w^{m4}/Y$ females. The results presented in

TABLE 2A

Tetrad analysis of total data from Table 1

Progeny from <u>XX</u> females					Progeny from <u>XXY</u> females				
Non-crossovers	Crossovers				Non-crossovers	Crossovers			
	Single	Double	Triple	Total		Single	Double	Triple	Total
4140	3503	356	2	8001	2918	2477	241	0	5636
991				12.4% E_0	682				12.1% E_0
2797	2797			69.9% E_1	1995	1995			70.8% E_1
350	700	350		17.5% E_2	241	482	241		17.1% E_2
2	6	6	2	0.2% E_3					— E_3
				100.0					100.0

TABLE 2B

Tetrad analysis of data from Table 1 omitting distal region (y^1 to f)

Progeny from <u>XX</u> females				Progeny from <u>XXY</u> females			
Non-crossovers	Crossovers			Non-crossovers	Crossovers		
	Single	Double	Total		Single	Double	Total
5298	2603	100	8001	3940	1657	39	5636
2795			34.9% E_0	2322			41.2% E_0
2403	2403		60.1% E_1	1579	1579		56.0% E_1
100	200	100	5.0% E_2	39	78	39	2.8% E_2
			100.0				100.0

TABLE 2C

Tetrad analysis of data from Table 1 omitting proximal region (wy to cv)

Progeny from <u>XX</u> females				Progeny from <u>XXY</u> females			
Non-crossovers	Crossovers			Non-crossovers	Crossovers		
	Single	Double	Total		Single	Double	Total
5478	2492	31	8001	3690	1916	30	5636
3017			37.7% E_0	1804			32.0% E_0
2430	2430		60.7% E_1	1856	1856		65.9% E_1
31	62	31	1.6% E_2	30	60	30	2.1% E_2
			100.0				100.0

Table 1 demonstrate that the normal Y chromosome does not alter total X crossing over in homozygous w^{m4} females when both the proximal and distal regions are included.

Table 2A presents the tetrad analyses of the crossover data from Table 1. The frequency of noncrossover tetrads (E_0 's) for those homozygotes for w^{m4} that lack a Y chromosome is virtually identical to the frequency of E_0 's for those homozygotes which carry the Y , i.e., 12.4% and 12.1%, respectively (columns 5 and 10). In fact, the entire tetrad distribution for w^{m4}/w^{m4} and $w^{m4}/w^{m4}/Y$ females is very similar (69.9% and 70.8% for E_1 's, respectively; 17.5% and 17.1% for E_2 's, respectively). Again, if the present crossover data were used but the distal region were omitted, tetrad analysis as illustrated in Table 2B, would show an apparent increase in E_0 's from 34.9% without the Y to 41.2% with the Y . Conversely, if the proximal region were omitted from the crossover data, tetrad analysis as shown in Table 2C would show an apparent decrease in E_0 's from 37.7% without the Y to 32.0% with the Y . Analysis of the *total* crossover data shows that the Y neither increases nor decreases the frequency of noncrossover X tetrads in this genotype.

As seen in Table 1, a decrease in proximal exchange is invariably observed in the presence of the normal Y . If this decrease arises from competitive X , X , Y pairing in the region where the X and Y share homology (STURTEVANT and

BEADLE 1936; R. F. GRELL 1962b), then MERRIAM's proposal to test the distributive pairing hypothesis in still another way should be a valid one. According to this test, when a nonhomologue is made available to the *Y*, and the success the nonhomologue achieves in pairing with the *Y* is calculated directly from the frequency with which the nonhomologue segregates from the *Y*, then the following two alternatives are possible:

(1) If the pairing of the *Y* and the nonhomologue (which is responsible for their eventual segregation behavior) precedes exchange, then crossing over in the proximal region of the *X* chromosomes should be increased to the extent that the *Y* associates with and segregates from the newly-provided nonhomologue and hence fails to associate with the *X* chromosomes.

(2) If the pairing of the *Y* and the nonhomologue (which is responsible for their eventual segregation behavior) follows exchange, then crossing over in the proximal region of the *X* chromosomes should not be altered by the association between the *Y* and the nonhomologue.

As noted earlier, MERRIAM assumed that the first alternative corresponds to the NOVITSKI model and the second one to the GRELL model. Actually, the first alternative differs radically from both models, and it is because of this difference that a genetic test is possible. The model which corresponds to the first alternative has been described by MERRIAM (1967), and in the present paper is referred to as the MERRIAM model. To clarify the critical difference between the MERRIAM model and the other two models, the main features of each are summarized below and represented diagrammatically in Figure 2.

The GRELL model (Figure 2A) postulates that homologues pair for exchange. This is called *exchange pairing*. The sole criterion for recognition between chromosomes at this time is homology. Following exchange pairing, exchange may or may not occur. Those homologues which undergo exchange remain associated and segregate regularly at anaphase I; those chromosomes which fail to undergo exchange form a group of univalents, called the *distributive pool*, whose composition varies from oocyte to oocyte depending on which chromosomes do not become exchange bivalents. Within the distributive pool a second kind of pairing now occurs, called *distributive pairing*. Nonhomologues as well as homologues may pair distributively since recognition at this time has been shown to depend on size and to be independent of homology (R. F. GRELL 1964a,b). Distributively paired chromosomes segregate at anaphase I.

The NOVITSKI "alternative" (NOVITSKI 1964) is shown in Figure 2B. A non-homologous association of all chromosomes prior to synapsis, perhaps as a chromocenter, is presumed to occur first and to place homologues in an advantageous position for synapsis. Then,

"... as pairs of homologs achieved a synaptic configuration, they would leave the chromocenter as independent bivalents. Those chromosomes unable to achieve this configuration (because of the absence of an exchange between homologs, or the absence of a homolog, or structural differences like inversions between homo-

FIGURE 2.—Meiotic pairing and segregation of the *X* chromosomes, a *Y* chromosome, and a single free *4*, as postulated by the models of GRELL, NOVITSKI, and MERRIAM.

logs, or attachment of homologs to a common centromere region, etc.) would remain, and *after* all synapsing pairs had removed themselves, would, by default, become paired with other chromosomes, homologs or not, which also were not involved in bivalent formation" [*italics mine*].

Comparison of Figures 2A and 2B disclosed that operationally the two models are identical. The chromocenter hypothesized in Figure 2B denotes no pairing or segregational commitment, since it represents an indeterminate association of the entire chromosomal complement. When, then, is the segregational decision between nonhomologues made in the NOVITSKI model? First, it is stated that nonhomologous pairing follows synapsis since it is only "*after* all synapsing pairs had removed themselves," that the remaining chromosomes may pair either homologously or nonhomologously. Second, synapsis must follow exchange since absence of exchange is given as a *cause* of absence of synapsis. Therefore, the chronological sequence is exchange, synapsis, and nonhomologous pairing. This means that in the NOVITSKI model, as in the GRELL model, the segregational commitment between nonhomologues occurs only after exchange and thus corresponds to the second alternative.

The MERRIAM model, which incorporates the alternative (1) is shown in Figure 2C. Its genetic predictions differ markedly from those of the other two. The essential difference lies in the intercalation of the final and definitive pairing step at a point *preceding* exchange. Pairing at this time may be between homologues or between nonhomologues, or a chromosome may be simultaneously involved with a homologue and a nonhomologue. In the last case, segregational consequences can result from nonhomologous pairing only if the homologues fail to undergo exchange. In this model, too, while pairing decisions precede exchange, segregational decisions must follow exchange. Once preexchange pairing occurs, however, a chromosome is irrevocably committed to a permanent alignment with

FIGURE 3.—MERRIAM model demonstrating that a pairing decision established prior to exchange is unalterable following exchange. (Modified from MERRIAM 1967.)

the chromosome or chromosomes of its choice (illustrated in Figure 3). Here we see that a nonhomologue, once paired, remains associated with the homologous bivalent until metaphase, whether exchange occurs or fails to occur in the bivalent. Thus, the MERRIAM model places severe restrictions upon the segregational consequences of nonhomologous pairing, since its potential is diminished to the extent that either participant subsequently becomes part of an exchange bivalent. By contrast, a mechanism which permits nonhomologous associations to be initiated after exchange, and restrictively between noncrossover chromosomes, provides the opportunity for segregational consequences to invariably follow such pairing. Herein lies one basis for a critical test of the two alternatives. This difference in the segregational consequences resulting from pre- or postexchange nonhomologous pairing will be examined later.

The difference in X exchange values predicted by a preexchange, as opposed to a postexchange, association between the Y and a nonhomologue was tested first by using the same competitor employed by MERRIAM, i.e., the multiply inverted second chromosome $SM1$. A segregation frequency of 92.7% between the Y and $SM1$ (Table 3, column 11) gave an association value of 85.4% (Table 3, column 11). This value describes, then, the percent restoration in proximal X values from $SM1$'s presence that is predicted by the preexchange pairing model; with the postexchange pairing model no restoration is anticipated. The observed values (Table 3, col. 3) show that crossing over in region 3 (the proximal region) is reduced from 22.6% to 19.4%, or 3.2 genetic crossover units, by the Y when the nonhomologue is absent. When the nonhomologue is present, the Y reduces proximal exchange from 25.2% to 21.4%, or 3.8 units. Thus, instead of an 85.4% restoration of proximal values with $SM1$, no restoration is observed. These results conform to those predicted by the postexchange pairing model. Moreover, the present studies show that total crossing over in XX and XXY females is the same, both for females carrying normal autosomes ($XX = 51.4\%$ and $XXY = 52.4\%$) and for females carrying $T(2;3) SM1;+$ ($XX = 63.9\%$ and $XXY = 65.6\%$) (Table 3, column 4). Tetrad analysis in the former case provides an E_0 frequency of 12.3% for XX and 12.9% for XXY females, in good agreement with the previous values based on larger numbers of 12.4% and 12.1% for XX and XXY females, respectively (Table 2). The increase in crossing over induced by $SM1$ is reflected in a decrease in E_0 frequency that is 4.7% for XX and 0 for XXY females. Since secondary nondisjunction in the latter case was found to be 1.4%, and since about 50% of w^{m4}/w^{m4} E_0 's appear to be diverted into nondisjunctionals by the Y , it is clear that some E_0 's must exist. The probable cause of the discrepancy is the failure to measure crossing over in the most proximal region between cv and w , where double exchanges are expected almost exclusively. If crossing over in this region is arbitrarily assumed to be about 1.5%, conversion of 1.5% of single crossovers into double crossovers would provide an E_0 frequency of about 2.9%, of which secondary nondisjunctionals would comprise about 50% (1.4/2.9). Naturally, a similar correction would be appropriate for each crossover study of w^{m4} homozygotes if the correct crossover value between cv and w were known.

TABLE 3
Crossing over values and tetrad frequencies for homozygous $\text{In}(1)w^{m4}$ in the absence and presence of nonhomologous Y, SMI association as calculated from the following crosses:

- (4) $w^{m4}, y/w^{m4}, cv \ v \ wy \ f/(N^{C-S})^+; +/+ \ \bar{q}\bar{q} \times \overline{XY}, y \ B/Y \ \delta\delta$
 (5) $w^{m4}, y/w^{m4}, cv \ v \ wy \ f/(N^{C-S})^+; \text{SMI}, a1^2 \text{Cy} \ cn \ sp/\text{I}(2:3)A, B1 \ \bar{q}\bar{q} \times \overline{XY}, y \ B/Y \ \delta\delta$

Mothers	Crossing over (%)†				Number	Tetrad analysis (%)				X Non-disjunction (%)	Y, SMI association (%)‡ (% segregation)
	Region 1 (y^1 to f)	Region 2 (f to wy)	Region 3 (wy to cv)	Total (y^1 to cv)		E_0	E_1	E_2	E_3		
Test 4 { $\overline{Y^{C-S}}; +/t_2 \ +/+$ 0, $+/t_2 \ +/+$	19.9 ↑ +4.1	13.1	19.4 ↓ -3.2	52.4	1889	12.9	70.8	15.5	0.9	7.2	
	15.8	13.0	22.6	51.4	2509	12.3	72.7	15.0	—	0.2	
Test 5 { $\overline{Y^{C-S}}; \text{I}(2:3)/\text{SMI}; +$ 0, $\text{I}(2:3)/\text{SMI}; +$	26.4 ↑ +3.2	17.8	21.4 ↓ -3.8	65.6	3835	0	70.0	29.0	1.0	1.4	85.4 (92.7)
	23.2	15.5	25.2	63.9	921	4.7	62.8	32.6	—	1.7	

* Designates $\underline{Y}$ present in some females; absent in sisters.

† Number to right of arrow indicates direction and amount of change induced by $\underline{Y}$ chromosome.

‡ $\underline{Y, SMI}$ association calculated from $\underline{Y, SMI}$ segregation by use of formula, $a = 1 - 2n$.

A complication inherent in the use of *SM1* as a nonhomologue is that two opposing modifications of crossing over are exerted on the region under study. On the one hand, the *Y*, presumably through competitive pairing, depresses proximal exchange and, on the other, *SM1* through the interchromosomal effect increases proximal exchange. If a conclusion is to be drawn about the action of either of the two modifiers from their collective action, it must be assumed that the effect each exerts in combination is equivalent to the effect each exerts alone.

To circumvent this complication, a second test was performed with a nonhomologue which presumably had no interchromosomal effect on the *X*. The chromosome chosen was a free second chromosome duplication, obtained through irradiation by E. H. GRELL, which carries the centromere and most of the proximal heterochromatin of chromosome 2 and probably all of the loci between brown (*bw*, 2-104.5) and the tip of 2*R*. The material capping 2*L* heterochromatin has not been identified, but it does not contain the locus of aristaless (*al*, 2-0.1) or of yellow (*y*, 1-0.0). Brain smears (Figure 4) show the duplication to be a small metacentric about one-third of the *X* chromosome in length.

The results obtained with *Dp(2;f)1* are given in Table 4. The observed segregation frequency of 94.5% between the *B^sY* and *Dp(2;f)1* translates into an association frequency of 89%. The latter value denotes, then, the expected restoration of proximal exchange values in the *w^{m4}* chromosome predicted by the preexchange pairing model when the *B^sY* and the duplication are both present and undergo nonhomologous association. Suppression of variegation of *w^{m4}* by *Dp(2;f)1* requires that a marked *Y* be used in this test. The proximal decrease with *B^sY* (Table 4, column 3) in the absence of the duplication was found to be only 0.7%, which does not conform to the more marked decreases obtained in all tests with the normal *Y*. With the numbers tested, 0.7% would constitute too small a difference to judge if any restoration is significant, but the observed decrease of 1.2% seen when both the *B^sY* and the duplication are present (Table 4, column 3) is approximately twice as great as with the *B^sY* alone and is in the direction opposite to that predicted by the MERRIAM model. As in the *SM1* situation, total crossing over is not altered by the *Y* whether the duplication is absent (*XX* = 53.8; *XXB^sY* = 54.3) or present (*XX;Dp* = 55.9; *XXB^sY;Dp* = 55.2).

FIGURE 4.—Brain smear showing *Dp(2;f)1*, indicated by arrow. (Courtesy E. H. GRELL.)

TABLE 4

Crossing over values and tetrad frequencies for homozygous $In(1)w^{m4}$ in the absence and presence of nonhomologous B^sY , $Dp(2;f)1$ association as calculated from the following crosses:

(6) $w^{m4}, y/w^{m4}, y^2/sc\ cv\ v\ wy\ f(B^sY)^*$; $sp^2/sp\ \dot{q}\dot{q}\ x\ al\ b\ c\ sp/al\ b\ c\ sp\ \dot{a}\dot{a}$

(7) $w^{m4}, y/w^{m4}, y^2/sc\ cv\ v\ wy\ f(B^sY)^*$; $Dp(2;f)1, sp^2/sp\ \dot{q}\dot{q}\ x\ al\ b\ c\ sp/al\ b\ c\ sp\ \dot{a}\dot{a}$

Mothers	Crossing over (%)†			Number	Tetrad analysis (%)				X Non-disjunction (%)	Y, Dp association (%)‡
	Region 1 (y^1 to f)	Region 2 (f to $w\dot{y}$)	Region 3 ($w\dot{y}$ to cv)		E_0	E_1	E_2	E_3		
Test 6 { $B^sY; sp^2/sp^2$ 0; sp^2/sp^2	18.0 16.5	13.1 13.3	23.3 24.0	1053 1233	12.3 13.9	66.9 64.7	20.9 21.4		6.7 0	
Test 7 { $B^sY; sp^2/sp^2/Dp(2;f)1$ 0; $sp^2/sp^2/Dp(2;f)1$	22.1 22.1	13.1 12.5	20.1 21.3	888 1402	14.4 7.7	64.4 74.0	17.6 17.1	3.6 1.1	6.5 1.1	89.0 (94.5)

* Designates B^sY present in some females; absent in sisters.

† Number to right of arrow designates direction and amount of change induced by the Y or the 2 duplication.

‡ Y, Dp association calculated from Y, Dp segregation by use of the formula $a = 1 - 2n$.

The third set of experiments made use of a dominantly marked fourth chromosome ci^D as the nonhomologous competitor. A prerequisite for the availability of the free 4 for nonhomologous pairing is the presence of a homozygous 3;4 translocation (see MATERIALS and METHODS). As shown in Table 5, column 3, in the absence of the extra 4, the Y was found to reduce proximal X exchange by 4.5 crossover units. With the preexchange pairing model, restoration of approximately 91%, corresponding to the calculated frequency of $Y,4$ association (Table 5, column 11) is expected in the presence of the competitor. Instead, a decrease of 4.3 crossover units is observed with the competitor (Table 5, column 3), demonstrating that no restoration occurs. Thus, predictions from the postexchange pairing model are again fulfilled.

An unexpected finding is the pronounced interchromosomal effect of $Dp(2;f)1$ and of the extra 4 on altering crossing over in w^{m4} homozygotes. The pattern mimics that seen with the normal Y (i.e., a marked proximal decrease and a marked distal increase in crossing over), thus raising the possibility that similar shifts in X exchange by the Y , previously ascribed to competitive X,X,Y pairing, may also be considered to be an interchromosomal effect. Still another interchromosomal effect on exchange that is observed with the homozygous 3;4 translocation, is the absence of the distal increase in exchange by the Y despite the occurrence of the proximal decrease (Table 5, column 1). As a result, in females carrying a Y and the homozygous 3;4 translocation, total X exchange is significantly reduced ($0;T/T = 53.1$; $Y;T/T = 47.1$). The presence of the extra 4 partially eliminates this effect.

The formula $P = B(1 - A)^*$ (R. F. GRELL 1962b) was used to estimate the required decrease in recombination in XXY compared to XX females if preexchange X,Y pairing were responsible for the E_o 's that became secondary exceptions. The predicted reduction was never observed and GRELL concluded that the findings were "incompatible with a model that postulates heterosynapsis prior to exchange as the source of the no-exchange X chromosomes that are recovered as exceptions." MERRIAM (1967) applied this formula to w^{m4} homozygotes and concluded that the data were not consistent with such a model, particularly since the reduction in exchange was not uniform, but, instead, showed a regional gradient which decreased distally. (Had the distal third been measured, the diminishing decrease would have been transformed into an increase.) Despite this conclusion, MERRIAM used his crossover data to demonstrate graphically that (1) crossing over is reduced in XXY mothers and (2) that recombination values for the proximal plus the medial region in XXY mothers are those expected by the formula $P = B(1 - A)$ when the BRIDGES bivalent-univalent model is assumed (MERRIAM 1967, Figure 4). Our present studies, however, show that when the distal third of w^{m4} is included (1) the difference in recombination between Y - and

* P = predicted frequency of crossing over in XXY females; B = observed frequency of crossing over in XX females; A = frequency of $X E_o$'s postulated from preexchange X,Y pairing, either as an XY bivalent and an X univalent according to BRIDGES, in which case $A = 2$ (secondary nondisjunction), or as an XYX trivalent according to COOPER, in which case $A =$ secondary nondisjunction.

TABLE 5

Crossing over values and tetrad frequencies for homozygous In(1)^{w^m4} in the absence and presence of nonhomologous Y,4 association as calculated from the following crosses:

- (8) $w^{m4}, y^1/w^{m4}, cv\ v\ wy\ f/(Y)^*$; $T(3;4)86D, bx^{34e}/T(3;4)86D, bx^{34e}\ \varnothing\ \varnothing \times \overline{XY}, y\ B/Y\ \delta\ \delta$
 (9) $w^{m4}, y^1/w^{m4}, cv\ v\ wy\ f/(Y)^*$; $T(3;4)86D, bx^{34e}/T(3;4)86D, bx^{34e}/cl^D\ \varnothing\ \varnothing \times \overline{XY}, y\ B/Y\ \delta\ \delta$

Mothers	Crossing over (%)†				Number	Tetrad analysis (%)				X Non-disjunction (%)	Y,4 association (%)‡
	Region 1 (y ³ to f)	Region 2 (j to wy)	Region 3 (wy to cv)	Total (y ³ to cv)		E ₀	E ₁	E ₂	E ₃		
Test 8 { Y; T/T 0; T/T	16.1↑ 15.7↓	11.8 13.7	19.3 23.8↓	47.1 53.1	3312 1768	14.8	76.4	8.6	0.2	7.7 0.2	
	+0.4 ↑		-4.5 ↓								
Test 9 { Y; T/T/cl ^D 0; T/T/cl ^D	21.3↑ 18.8↓	12.8 13.1	16.5 20.8↓	50.6 52.7	1020 2376	8.8	81.4	9.8	...	3.5 0.5	90.8 (95.4) ...
	+3.1 +2.5 ↑		-4.3 ↓	-3.0 ↓							

* Designates Y present in some females; absent in sisters.

† Number to right of arrow designates direction and amount of change induced by the Y chromosome or the extra 4.

‡ Y,4 association calculated from Y,4 segregation by use of the formula $a = 1 - 2n$.

no Y -bearing sisters disappears, and (2) the observed values for XXY females are in marked excess of those predicted by the formula $P = B(1 - A)$. This is seen in Figure 5, which presents the recombination values from w^{m4}/w^{m4} females (y to cv) as a function of the values from their XXY sisters. The points, except for No. 8, conform well to the straight line denoting $XX = XXY$ recombination. Females corresponding to No. 8 carry both the Y and the homozygous 3;4 trans-

FIGURE 5.—Comparison of fit of two different plots to a straight line. (a) Observed crossing over in XXY females plotted against observed crossing over in XX females; (b) Observed crossing over in XXY females plotted against expected crossing over in XXY females from formula $P = B(1-A)$.

● = observed crossing over in XX ;

Δ = expected crossing over in XXY . Genotypes and data for tests 1–3 are given in Table 1; for tests 4 and 5, in Table 3; for tests 6 and 7, in Table 4; and for tests 8 and 9, in Table 5.

location and, as noted previously, show the proximal decrease but no distal increase so that total crossing over is reduced. In addition Figure 5 presents the values predicted for $w^{m4}/w^{m4}/Y$ females by the formula $P = B(1 - A)$ plotted as a function of the present observed values for $w^{m4}/w^{m4}/Y$ females. Most points lie distant from the predicted slope and in every case the points lie above the line, indicating that observed values always exceed predicted values.

MERRIAM subjected his recombination data to tetrad analyses, plotted secondary nondisjunction as a function of the difference in E_0 's from XX and XXY mothers (MERRIAM 1967, Figure 5), and claimed a linear relationship. Again, it must be emphasized that neglect of the distal third of the chromosome precluded determination of the actual frequency of E_0 's, upon which secondary nondisjunction has been shown repeatedly to be dependent (STURTEVANT and BEADLE 1936; R. F. GRELL 1962b). A plot for the present data is given in Figure 6. No linear relationship is found, and the points appear scattered about the abscissa, with four of the nine points lying below the abscissa and five above. Moreover, the frequency of secondary nondisjunction is approximately the same whether the points fall above or below the abscissa, showing that slight differences in E_0 frequencies from XX and XXY sisters cannot account for the differences in X nondisjunction observed in these sisters.

FIGURE 6.—Absence of relationship between percent secondary nondisjunction and XXY E_0 's. Genotypes and data for 1–3 are given in Table 1; for 4 and 5, in Table 3; for 6 and 7, in Table 4; and for 8 and 9, in Table 5.

FIGURE 7.—Rise in X nondisjunction with increase in XXY E_0 's contrasted with absence of such a rise with increase in XX E_0 's. In $\odot^9$, $\odot^7$, $\odot^5$, a free 4, a 2 duplication, and *SM1*, respectively, are present, which, like a Y , act to induce distributive nondisjunction of XX E_0 's. $\odot$ = primary nondisjunction; $\blacktriangle$ = secondary nondisjunction. Genotypes and data for 1–3 are given in Table 1; for 4 and 5, in Table 3; for 6 and 7, in Table 4; for 8 and 9, in Table 5.

A more meaningful approach is given in Figure 7, where X nondisjunction is plotted as a function of E_0 's for both XX and XXY sisters. Secondary nondisjunction now displays a linear relationship with the frequency of E_0 's, as seen from the proximity of $\blacktriangle$ points to a line with a slope of 2. Such a line would correspond to 50% of X E_0 's becoming nondisjunctionals in the presence of the Y . By contrast, primary nondisjunction points ($\odot$) cluster close to the ordinate ($=0$) although E_0 frequencies are as high in these XX females as in their XXY sisters. In the absence of a nonhomologue for distributive pairing with X E_0 's ($\odot$ points 1,2,3,4,6,8), primary nondisjunction values are seen to vary from 0 to 0.2%;

with the smallest nonhomologue ci^D present ($\odot$ point 9) the value increases to 0.5%; with the intermediate nonhomologue $Dp(2;f)1$ ($\odot$ point 7) it is 1.1%; with the largest nonhomologue $SM1$ ($\odot$ point 5) it is 1.7%. It is evident that a size-dependency, characteristic of recognition between nonhomologues at distributive pairing, is here again displayed for each of the three nonhomologues with the $X E_0$'s. Of particular interest is the proximity of $SM1$ ($\odot$ point 5) to the hypothetical line for secondary nondisjunction, indicating that $SM1$ approximates the Y in this respect. Since X nondisjunction induced by $SM1$, by $Dp(2;f)1$ and by ci^D differs in no fundamental respect from that induced by a Y chromosome, it is proposed that the term "distributive nondisjunction" serve as a general term to describe nondisjunction of homologues that results from distributive pairing of heterologues. The term "secondary nondisjunction," because of its long usage, could be retained to describe the particular type of distributive nondisjunction that results from distributive pairing between the X and Y chromosomes.

The validity of the tests reported above hinges on the assumption that the depression in X exchange by the Y marks the position of the Y at the time of exchange. This assumption has been weakened by the finding that a free second chromosome duplication and a single free fourth chromosome cause similar depressions. The present data are, however, susceptible to a different kind of analysis that requires no such assumption. In this test, two different estimates of Y association frequencies can be made, one for the pre- and one for the post-exchange pairing model. The difference in estimates arises because associations established between nonhomologues before exchange frequently involve a certain fraction of potential crossovers, and these associations can have no segregational consequences. By contrast, associations established after exchange avoid this contingency by selectively involving only noncrossovers. Thus, if pairwise associations between a Y and either the X or a nonhomologue are irreversibly established before exchange, as assumed by the MERRIAM model, we can estimate the frequency with which the Y must be involved with the X , on the one hand, and with the nonhomologue, on the other, to account for the observed segregations. Similarly, we can estimate this frequency for the distributive model. In both cases, we will find whether this value falls within the permissible limit of 100%. If both values conform to this limit, this particular test would not distinguish between them; but should results from the former but not the latter model significantly exceed this limit, a distinction could be made.

The results from the above test are presented in Table 6. If segregation pairing decisions between nonhomologues are made before exchange, the frequency of associations between the X chromosomes and the Y is calculated as the percent of X nondisjunction divided by the percent of $X E_0$'s. This fraction represents the amount of X,Y pairing that must occur before exchange if the Y is to divert the required percent of $X E_0$'s into nondisjunctionals after exchange. This is so because the Y cannot identify potential noncrossovers before exchange, yet only noncrossovers can become secondary exceptions. Two different X,Y association frequencies have been calculated which correspond to the trivalent model and the

TABLE 6
Total frequencies of *Y* association with preexchange vs. postexchange segregational pairing models

Preexchange		Postexchange	
Y,X,X association =	trivalent = $\frac{\% X \text{ n.d.}}{\% X E_0's}$	Y,X,X association =	trivalent = $\% X \text{ n.d.}$
	bivalent = $2 \left(\frac{\% X \text{ n.d.}}{\% X E_0's} \right)$		bivalent = $2(\% X \text{ n.d.})$
nonhomologous Y association	$= \frac{1 - 2(\% \text{ n.d.})}{\% E_0's \text{ nonhomologue}}$	nonhomologous Y association	$= 1 - 2(\% \text{ n.d.})$
$SM1 \left\{ \begin{array}{l} Y, SM1 \\ Y, X, X \end{array} \right\}$	$\frac{XYX}{XY + X} > 85\%$	$SM1 \left\{ \begin{array}{l} Y, SM1 \\ Y, X, X \end{array} \right\}$	$\frac{XYX}{XY + X} > 85\%$
	$\frac{85\%}{<100\%} = \frac{85\%}{>85\%}$		$\frac{XYX}{XY + X} = \frac{85\%}{85\%}$
	$\frac{1.4\%}{0^*} = \frac{1.4\%}{\sim 40\%}$		$\frac{XYX}{XY + X} = \frac{1.4\%}{2.8\%}$
Total Y association	$\frac{>125\%}{>165\%}$	Total Y association	$\frac{86.4\%}{87.8\%}$
$Dp(2;f)1 \left\{ \begin{array}{l} Y, Dp2 \\ Y, X, X \end{array} \right\}$	$\frac{89\%}{100\%} = 89\%$	$Dp(2;f)1 \left\{ \begin{array}{l} Y, Dp2 \\ Y, X, X \end{array} \right\}$	$\frac{89\%}{89\%} = 89\%$
	$\frac{6.5\%}{14.4\%} = 45\%$		$\frac{6.5\%}{13\%} = 6.5\%$
	$\frac{90\%}{179\%}$		$\frac{102\%}{102\%} = 102\%$
Total Y association	$\frac{134\%}{179\%}$	Total Y association	$\frac{95.5\%}{102\%}$
$4 \left\{ \begin{array}{l} Y, 4 \text{ association} \\ Y, X, X \text{ association} \end{array} \right\}$	$\frac{91\%}{100\%} = 91\%$	$4 \left\{ \begin{array}{l} Y, 4 \text{ association} \\ Y, X, X \text{ association} \end{array} \right\}$	$\frac{91\%}{91\%} = 91\%$
	$\frac{3.5\%}{8.8\%} = 40\%$		$\frac{3.5\%}{94.5\%} = 3.5\%$
	$\frac{80\%}{171\%}$		$\frac{98\%}{98\%} = 98\%$
Total Y association	$\frac{131\%}{171\%}$	Total Y association	$\frac{94.5\%}{98\%}$

*An *X* nondisjunction value of 1.4% requires a minimal frequency of 1.4% *X E*'s, in which case 100% *XYX* trivalent or 200% *XY + X* bivalent, univalent associations must occur. Instead, values of 40% and 80% have been used, which are in line with those found with *Dp(2;f)1* and chromosome 4.

bivalent-univalent model. The latter requires twice the frequency of X,Y pairing as the former to account for the same frequency of X nondisjunction. In a similar way, the frequency of Y , nonhomologue association required by the MERRIAM model is calculated by dividing the association value ($a = 1 - 2n$) by the frequency with which the nonhomologue is a nonexchange tetrad (E_0). In the cases of $Dp(2;f)1$ and of chromosome 4, the E_0 frequencies approximate 100%; in the case of $SM1$ the value is less than 100%, since an undetermined number of cross-overs between $SM1$ and the translocated 2 are produced but only the noncrossover strands from such tetrads are recovered.

Inspection of Table 6 shows that if segregational pairing decisions precede exchange (left half), the total frequencies of Y pairing required with a trivalent XYX model are $> 125\%$ for $SM1$, 134% for $Dp(2;f)1$ and 131% for the free 4. If the bivalent-univalent model is postulated, the corresponding values are $> 165\%$, 179% , and 171% , respectively. In either case, all values are greatly in excess of the 100% limit.

Alternatively, the values calculated for a model which assumes that segregational pairing decisions are made after exchange is shown in the right half of Table 6. In this case X,Y association values are identical to secondary nondisjunction values, using an XYX trivalent model, and twice nondisjunction values using an $XY-X$ bivalent-univalent model. Association values for the Y and the nonhomologue are directly equivalent to the association value calculated from the expression $a = 1 - 2n$. Total Y -association turns out to be 86.4% in the case of $SM1$, 95.5% with $Dp(2;f)1$, and 94.5% with the free 4 if an XYX trivalent is postulated. Corresponding values for a bivalent-univalent model are 87.8% , 102% , and 98% , respectively. None of these values significantly exceeds the maximal limit.

DISCUSSION

Genetic tests to determine if homologous and nonhomologous pairing are competitive may take several forms. A direct test compares exchange frequencies between a pair of homologues when one member of the pair does, and when it does not, participate in nonhomologous associations. The repeated failure to observe a difference in exchange in the two situations, coupled with the restriction of nonhomologous associations to noncrossovers, prescribed the meiotic sequence defined by the distributive pairing model. In the original studies (R. F. GRELL 1962a) comparisons were made for exchange frequencies in the distal half of a chromosome; subsequent studies have measured exchange throughout the chromosome.

A less direct method, but a valid one, compares the effect of a triplication which does not undergo exchange itself but which pairs competitively to reduce exchange between homologues, both when the triplication is involved and when it is uninvolved in nonhomologous associations. MERRIAM's test with w^{m4} falls into this category. The Y represents the triplication-bearing chromosome, and shared homology between the Y and the proximal heterochromatin of the X is presumed to be responsible for the reduction in proximal X exchange. As the nonhomologue

TABLE 7A

Comparison of MERRIAM and GRELL crossover values (f to cv) with and without a Y chromosome in the presence and absence of SM1; +/T(2;3)A

Genotype	MERRIAM			GRELL		
	Medial (f-m)	Proximal (m-cv)	Total (f-cv)	Medial (f- <i>wy</i>)	Proximal (<i>wy</i> -cv)	Total (f-cv)
0; +/+; +/+	20.4	16.8	37.2	13.0	22.6	35.6
Y; +/+; +/+	19.6	13.1	32.7	13.1	19.4	32.5
	— 0.8	— 3.7	— 4.5	+ 0.1	— 3.2	— 3.1
0; SM1; +/T(2;3)	22.3	22.0	44.3	15.5	25.2	40.7
Y; SM1; +/T(2;3)	24.0	19.2	43.2	17.8	21.4	39.2
	+ 1.7	— 2.8	— 1.1	+ 2.3	— 3.8	— 1.5

which competed with the *X*'s for association with the *Y*, MERRIAM chose a multiply inverted second chromosome, *SM1*. As with his other tests, the distal portion of the *X* was omitted from study. His conclusion was drawn from the entire portion of the *X* that he measured, which included the medial region as well as the proximal region. If the present data were treated so as to correspond to MERRIAM's data (i.e., if the distal region were omitted and the medial region included) a decreased effect of the *Y* on *X* exchange would here too be observed in the presence of *SM1* (Table 7A, columns 3 and 6). It may be seen, however, that in both cases the decreased effect is not caused primarily by a restoration of proximal values (*m* to *cv* or *wy* to *cv*), but by an increase in medial values (*f* to *m* or *f* to *wy*) in the combined presence of the *Y* and *SM1*;+/T, an increase that fails to occur with the *Y* alone. This increase is probably another manifestation of the interchromosomal effect. In any case it cannot be attributed to decreased *Y* pairing in the presence of *SM1*, since removal of the *Y* by *SM1* is not expected to increase crossing over in excess of what it was in the absence of the *Y*. When the distal region is included (Table 7B, column 4) it becomes apparent that total exchange

TABLE 7B

Effect of adding distal region to GRELL values

Genotype	GRELL			
	Distal (<i>y</i> ^L -f)	Medial (f- <i>wy</i>)	Proximal (<i>wy</i> -cv)	Total (<i>y</i> ^L -cv)
0; +/+; +/+	15.8	13.0	22.6	51.4
Y; +/+; +/+	19.9	13.1	19.4	52.4
	+ 4.1	+ 0.1	— 3.2	+ 1.0
0; SM1; +/T(2;3)	23.2	15.5	25.2	63.9
Y; SM1; +/T(2;3)	26.4	17.8	21.4	65.6
	+ 3.2	+ 2.3	— 3.8	+ 1.7

is unaffected by $Y, SM1$ associations. Moreover, if attention is confined to the proximal region, where the significant decrease by the Y is encountered, then one finds comparable decreases by the Y whether $SM1$ is present or absent. Thus, weighted averages of the proximal decrease by the Y observed by MERRIAM for the two genotypes which lack $SM1$ ($+/+$, $+/+$ and $T(2;3)/+;$) and the two which carry $SM1$ ($SM1/+;$ $+/+$ and $SM1; +/T(2;3)$) turn out to be 3.8 crossover units; and 3.9 crossover units, respectively. Similarly, the present results reveal a proximal X reduction by the Y between $w\gamma$ and cv of 3.2 crossover units when $SM1$ is absent and a reduction of 3.8 crossover units when $SM1$ is present. The failure of nonhomologous associations between the Y and $SM1$ to alter the effect of the Y on w^{m4} exchange is demonstrated by total exchange values of 52.4% for $w^{m4}/w^{m4}/Y$ females and 51.3% for w^{m4}/w^{m4} females in the absence of $SM1$; which are increased through the interchromosomal effect to 65.6% for $w^{m4}/w^{m4}/Y$ females and 64.0% for w^{m4}/w^{m4} females in the presence of $SM1$. Comparisons of total exchange values cannot be made in MERRIAM's tests because the distal region was omitted.

The conclusion that associations between the Y and a nonhomologue do not influence the ability of the Y to alter X exchange finds further corroboration in two additional tests. Here the nonhomologues that were utilized were a free second chromosome duplication in one case and a free fourth chromosome in the other. The proximal X reduction between $w\gamma$ and cv is 0.7 crossover units in the presence of the B^sY alone and is 1.2 crossover units when the 2 duplication is added despite a calculated B^sY , 2 Dp association frequency of 89%. In the third case, with a free fourth chromosome as the nonhomologue, the proximal reduction by the Y between $w\gamma$ and cv was found to be 4.5 crossovers units in the absence of the four and 4.3 crossover units in its presence, while $Y,4$ association frequency was calculated as 91%. For all situations tested, then, the results conform to those predicted by the distributive pairing model.

Unexpectedly, the studies with the different nonhomologous competitors disclosed that changes in X crossing over, of the same pattern and magnitude as those caused by the Y , occur with both a free four and with a free second chromosome duplication. Proximal reductions in X exchange for the region between $w\gamma$ and cv with a normal Y chromosome, with an extra free fourth chromosome, and with a free 2-duplication are 3.3, 3.8, and 2.7 crossover units, respectively; distal increases between γ and f are 4.1, 3.1, and 5.6 crossover units, respectively. Thus, the assumption which served as the basis for the MERRIAM test [i.e., alterations in X exchange by the Y result from competitive X,Y pairing in their region of shared homology (STURTEVANT and BEADLE 1936; GRELL 1962b)] is rendered less plausible.

Another indirect test, but one which is free of the ambiguity encountered in the present version, has previously been reported (GRELL 1967). In this case the complication arising from the choice of a triplication with ill-defined homology (i.e., the Y for the X) was avoided by employing a series of X duplications whose homology for the distal tip of X was rather precisely circumscribed by both genetic and cytological analyses. The experimental situation possessed an additional advantage in that the depression in X exchange imposed by the X duplica-

tion in the region of homology between γ and w attained values as great as 67% as compared to about 20% in the present tests. Under these conditions it was found that whether or not the duplications participated in nonhomologous associations with a free fourth chromosome, the depression in distal X exchange remained unaltered. For example, $Dp\ z^9$ reduced crossing over between γ and w from a normal value of $1.57\% \pm 0.12$ to $0.52\% \pm 0.13$, or a 67% reduction. The addition of a free four, from which z^9 was found to segregate 97%, did not alter the effect of z^9 on exchange between γ and w , which now had a value of $0.51\% \pm 0.14$. This indirect test therefore confirms what had previously been established by direct tests. Segregational pairing between nonhomologues must follow exchange and must constitute an independent pairing event possessing its own special properties.

Finally, the present data have been subjected to a different kind of analysis. The MERRIAM model, based on a preexchange pairing decision, and the GRELL and NOVITSKI models, based on a postexchange pairing decision, provide different estimates of the total Y association that is required to account for the segregational commitments of the Y with the X chromosomes and with the nonhomologue. For the preexchange decision model, calculations (Table 6, columns 1, 2) show in all three cases that the sum of Y involvement with the X chromosome and with the nonhomologue would significantly exceed the maximal permissible frequency of 100%. This is true for the trivalent as well as the bivalent-univalent model of X,Y,X pairing. By contrast, for the postexchange decision model, calculations of these frequencies (Table 6, columns 3, 4) show that the sum of Y involvement with the X and with the nonhomologue approaches but does not significantly exceed the 100% maximum. Further, no value less than 86% of the maximum is observed, indicating, as might be expected from the availability of the nonhomologue following exchange, that the Y is associated with the nonhomologue or the X most of the time. The prime determinant of total Y association is the availability of the nonhomologue. In the case of $SM1$, exchange between the multiply inverted 2 and its homologue in the translocation to produce crossover tetrads which cannot enter the pool but from which only noncrossover strands are recoverable, accounts for the depressed frequency of $Y,SM1$ associations (85%); when the nonhomologue is the 2 duplication or chromosome 4, neither of which are part of crossover tetrads, nonhomologous associations are increased.

The availability of only two nonhomologues in the distributive pool assures that these two chromosomes will segregate with a high frequency ($\sim 90\%$ to 99.9%) regardless of their size. How high that frequency will be has been shown to be positively correlated with their similarity in size (R. F. GRELL 1964a,b). A size-dependency is again displayed in the distributive pairing of nonhomologues in the present experiments. As seen in Figure 7, a comparison of the fraction of $X\ E_0$'s from XX mothers induced to undergo nondisjunction by the 4, the 2 duplication, and $SM1$ arranged in increasing order of size similarity to the X , are $7\% \left(\frac{0.5\ X\ n.d.}{7.3\ X\ E_0's} \right)$, $14\% \left(\frac{1.1\ X\ n.d.}{7.7\ X\ E_0's} \right)$, and $36\% \left(\frac{1.7\ X\ n.d.}{4.7\ X\ E_0's} \right)$, respectively.

The effect of the Y on exchange in w^{m4} homozygotes is of interest for an addi-

tional reason. A "generalization" stating that exchange is always equal in XX and XXY females and attributed by MERRIAM (1967, p. 415) to R. F. GRELL (1962b), was claimed by MERRIAM to be untrue for females homozygous for inversions whose proximal breakpoint lies distal to the nucleolar organizer in proximal heterochromatin, such as w^{m4} . Concerning the "generalization," point 5 of the summary of the GRELL (1962b) paper establishes that the converse conclusion was reached,

"The effect of a Y chromosome on crossing over in X -inversion heterozygotes is correlated with the position of the inversion. In XXY females heterozygous for distal inversions a decrease is seen in total crossing over caused by the elimination of the proximal intrabrachial effect. In XXY females heterozygous for proximal or medial inversions a distal increase and, hence, a net increase in crossing over is seen."

Since reliable estimates of total crossing over in w^{m4} homozygotes in the presence and absence of a Y (Table 1) show that crossing over is alike in the two situations, it follows that even if such a generalization had existed, the case of w^{m4} would support rather than disprove it.

The ability of the extra 4 or the 2 duplication to mimic the Y in altering X exchange adds still another class of genotypic changes to the presently recognized array of interchromosomal effects. Although the mechanism through which such effects are produced is not understood, recent evidence tends to favor a physiological rather than a mechanical interpretation (RAMEL 1962). In the present case, however, the possibility of a mechanical effect, exerted perhaps through a chromocenter or a bouquet and dependent on regions of shared homology in the proximal heterochromatin of all members of the genome, may be considered. The 4 and the 2 duplication, like the Y , might then reduce X exchange through competitive exchange pairing in the proximal regions, without affecting segregational pairing decisions which, as shown above, must follow exchange.

It is immediately apparent that should proximal heterochromatin represent the repository of duplicated genetic material, this material can be neither qualitatively nor quantitatively equivalent between heterologues. As examples, the nucleolar organizer region found in the proximal heterochromatin of the X and Y is absent from the autosomes; the proximal heterochromatin of the X alone is 3-4 times the entire length of chromosome 4 including any proximal heterochromatin present therein; size differences are also apparent between the proximal heterochromatin of the X and that of chromosomes 2 and 3; and in the latter cases the heterochromatin is distributed on either side of the centromere, whereas in the X it lies to the left of the centromere. Although the possibility that these regions may share some homology cannot be excluded, it seems clear that the heterochromatin of homologues must possess greater homology than that found between heterologues, and that competition should favor pairing between homologues in heterochromatic as well as in euchromatic regions, providing intimate pairing actually occurs in the former case (see below). On the other hand, should proximal pairing turn out to be noncompetitive, it becomes difficult to imagine that crossing over could be mechanically altered by the addition of a Y , a 4 or a 2 duplication to a chromocenter.

Further, cytological studies provide no support for the supposition that a pre-exchange chromocenter or a bouquet stage exist in the *Drosophila* oocyte, which would permit heterochromatic pairing of heterologues at the same time that euchromatic pairing occurred between homologues. In fact, GUYÉNOT and NAVILLE (1933) are unequivocal on this point. They describe the earliest oocytes as follows:

"When the last gonial division had ended, the small group of 16 cells shows small nuclei (only 3–4 μ in diameter), most of them clear, rather crowded against one another, and each with a clearly visible nucleolus. But, among these elements, two are immediately distinguishable by the presence of three stained bodies, more or less regular in form and corresponding without any doubt to the three pairs of large chromosomes. In fact, it is a matter here of prochromosomes closely united two by two by prosynapsis and evidently corresponding to the figures observed by one of us at the beginning of the evolution of the oöcyte in *Calliphora*. . . . The prochromosomes pass then to a diffuse state during which the heterochromosomes alone retain their contracted state (heteropycnotic) . . ." (GUYÉNOT and NAVILLE *op. cit.* p. 221).

And then,

"The very faint chromatic network, resulting from the transformation of the prochromosomes, gives rise to very fine chromatic filaments, corresponding to the leptotene stage . . . These filaments are joined two by two for part of their length, thus attaining a parasynapsis . . . This first phase, which requires a decontraction of the prochromosomes, has been followed with more detail in *Calliphora* by NAVILLE (1932). He only observed this in the young cysts during their formation . . . The paired state of the leptotene loops seem very precocious and may even be only the consequence of the union two by two of the homologous prochromosomes. During this amphitene [= zygotene or synaptotene] state, the movement to the center does not manifest itself so that there is no production of a true bouquet. Similarly in *Calliphora*, we have never observed any contraction or synzinesis." (*ibid.* p. 221).

It should be further noted that the chromocenter association described by KING, RUBINSON and SMITH (1956), and referred to by NOVITSKI (1964) is in fact an association of all the chromosomes, known as the *karyosome*. Karyosome stages represent a period *following* crossing over and preceding diakinesis corresponding probably to a modified diplotene stage (KING, RUBINSON and SMITH 1956).

As an alternative to a mechanical effect, exerted through a bouquet or chromocenter, the interchromosomal effect of the *Y*, the 2 duplication, and the 4 may be assumed to be physiological in nature. Too little is known to speculate on the specificities of such action, but an analogous case would seem to be found in the induction of fourth chromosome crossing over through the use of diplo-4 triploids (STURTEVANT 1951). Here it becomes exceedingly difficult to explain the effect as other than a physiological one, attributable, perhaps, to a change in the euchromatic-heterochromatic balance of the genome, an explanation which might also serve in the present case.

The possibility that the *Y* alters *X* exchange through physiological means provides an explanation for certain puzzling results obtained from *X*-duplication studies (GRELL 1967). The *X* duplications carried not only different lengths of *X*

euchromatin but also different lengths of X heterochromatin, including in one case, the nucleolar organizer and heterochromatin distal to it. Nonetheless, the depression in X exchange by the duplications was found to be entirely correlated with the amount of distal- X homology present and was unrelated to the length of heterochromatin. In other words, proximal, heterochromatic pairing was apparently not competitive with distal, euchromatic pairing. It is well known that heterochromatin rarely undergoes exchange (BROWN 1940; BAKER 1958; ROBERTS 1965). This result suggested that heterochromatin may fail to undergo exchange pairing as well as exchange. In contradiction to this possibility stood the Y chromosome's well-recognized effect of depressing proximal crossing over, which was assumed to arise through competitive X,X,Y , exchange pairing in the heterochromatic region. If the Y effect should turn out to be physiological rather than mechanical in origin, the contradiction becomes resolved.

SUMMARY

A reexamination of crossing over in w^{m4}/w^{m4} and $w^{m4}/w^{m4}/Y^{c-s}$ females, including the distal third (γ to f) of the genetic length of the X chromosome has failed to confirm the conclusions reached by MERRIAM (*op. cit.* 1967). Present studies have revealed that a significant proximal decrease (3.3 crossover units) is compensated for by a significant distal increase (4.1 crossover units) and that total exchange is unaltered by the Y ($w^{m4}/w^{m4} = 52.7 \pm 0.6$; $w^{m4}/w^{m4}/Y^{c-s} = 52.5 \pm 0.7$). The frequency of noncrossover tetrads (E_0 's) for w^{m4}/w^{m4} (12.4%) and for $w^{m4}/w^{m4}/Y^{c-s}$ females (12.1%) is unchanged by the Y .—MERRIAM's indirect test to determine the time of initiation of segregational pairing between nonhomologues, employing in the present version three different nonhomologous competitors for the Y , including $SM1$, a free 2 duplication, and a free fourth chromosome, has shown, in each case, that the depression in proximal X exchange, presumably arising from competitive Y pairing with the X 's, is not restored by high frequencies of Y pairing with the nonhomologue (85–91%). The result is consistent with the distributive model, which postulates that segregational pairing commitments between nonhomologues are initiated after exchange. It is incompatible with preexchange initiation.—Doubt is cast on the validity of MERRIAM's test by the finding that a 2 duplication and an extra free 4 cause proximal decreases and distal increases comparable to those observed with the Y . The possibility that the Y effect is an interchromosomal one which is physiological in nature is discussed.—A different kind of analysis, utilizing exchange and segregation data but requiring no additional assumption, concurs with previous results from direct and indirect tests in demonstrating that the inception of segregational pairing between nonhomologues must follow exchange.—A size dependency, characteristic of recognition between chromosomes at distributive pairing, is again encountered between the X and $SM1$, the X and the 2 duplication, and the X and the free 4.—It is proposed that heterochromatic regions, which rarely undergo exchange, may fail to do so because they rarely undergo pairing for exchange.

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