THE INITIATION OF NONHOMOLOGOUS CHROMOSOME PAIRING BEFORE EXCHANGE IN FEMALE *DROSOPHILA MELANOGASTER*[1]

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THE regular association of nonhomologous chromosomes, in certain genotypes, may be inferred from their regular segregation from each other in meiosis of female *Drosophila melanogaster* (SANDLER and NOVITSKI 1956; COOPER, ZIMMERING and KRIVSHENKO 1955; GRELL 1957, 1959). Such nonhomologous pairing is competitive with regular homologous pairing for partners at disjunction and leads to nondisjunction of homologues. It has been determined that nonhomologous pairing is limited to elements from no-exchange tetrads but that the occurrence of nonhomologous pairing does not increase the incidence of no-exchange tetrads. Thus, nonhomologous pairing does not normally compete with homologous pairing for partners at the time of exchange but occurs only among existing no-exchange tetrads. (GRELL [1962a,b] and STURTEVANT and BEADLE [1936] determined that the frequency of secondary exceptions from XXY females "is dependent on the occurrence of noncrossover tetrads, rather than the reverse".) These observations specify that the final decision as to which chromosomes disjoin from each other follows exchange but do not specify when the initiation of nonhomologous association occurs relative to exchange and, in particular, whether it and the initiation of pairing for exchange occur together or sequentially. There are two models of the meiotic sequence which are consistent with the observations. GRELL (1962a) has hypothesized that there are regularly two pairing events in meiosis. First homologous chromosomes pair for exchange. Second, following exchange, nonrecombinant chromosomes enter a distributive pool from which partners, which may be homologous or nonhomologous, are chosen for distributive pairing and disjunction. NOVITSKI (1964), on the other hand, postulates a single association of chromosomes during meiosis, perhaps by chromocenter formation, in which nonhomologous associations are not competitive with exchange in homologous regions. Exchange ensures the proper orientation of tetrads at disjunction on the NOVITSKI sequence and only no-exchange tetrads are subject to the influence of previously determined chromocentral associations. These sequences, with regard to pairing for disjunction, are diagrammed in Figure 1 where homologous chromosomes are represented by identical open figures and a nonhomologue by the solid figure. Both models yield the same expectations of regular disjunction

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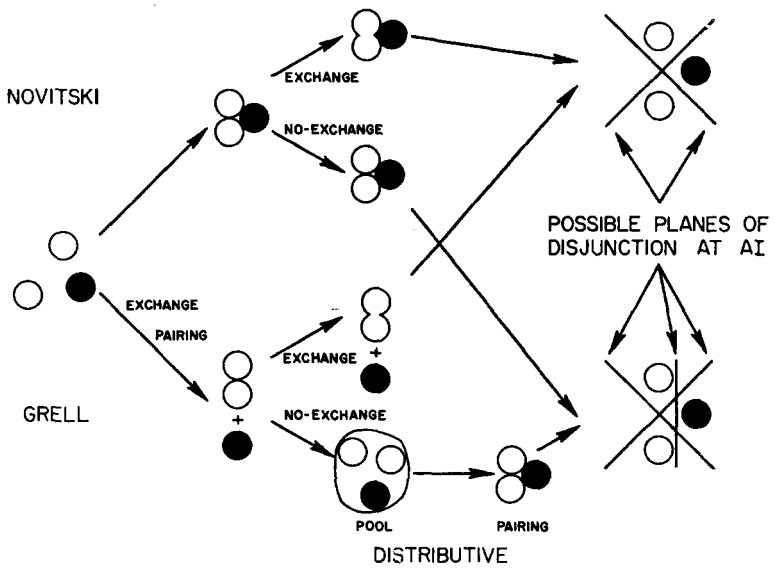

FIGURE 1.—The GRELL and NOVITSKI alternative sequences of meiosis, explained in text, with homologues generally represented by open circles and nonhomologues generally represented by the solid circle.

of homologues from exchange tetrads as well as possible nondisjunction of homologues from no-exchange tetrads.

The report is concerned with distinguishing between these two sequences of meiosis and determining the time of nonhomologous pairing. Such a distinction is possible because of the behavior of certain X-chromosome inversions when homozygous in XXY females. Homozygous inversions should have no structural difficulty in pairing and ought to exhibit normal exchange values and, hence, undergo secondary nondisjunction with the same frequency as normal X chromosomes (STURTEVANT and BEADLE 1936). OKSALA (1958) has reported, however, that XXY females homozygous for *In(1)w*^{m4} yielded 17.2% exceptional gametes, i.e., 2 to 3 times higher than that observed with normal chromosomes. This implies either that crossing over is less frequent than normal in *In(1)w*^{m4} homozygotes, or that the Y chromosome reduces the frequency of exchange in *In(1)w*^{m4}/*In(1)w*^{m4}/Y females, or that some exceptions are produced from exchange tetrads in *In(1)w*^{m4} homozygotes. The results indicate that the second alternative is correct; this response to the presence of a Y chromosome seems to be confined to homozygotes for inversions with one breakpoint in the proximal heterochromatin distal to the nucleolar organizer.

This reduction in recombination suggests that the Y chromosome is associated with the X's at the time of exchange. The results of OKSALA also show that a complex inversion-second chromosome competes successfully with the X's for the Y chromosome as a disjunctive partner. This makes available a test, ordinarily not possible, to measure the effect of competition between the X's and an inversion-bearing second chromosome for nonhomologous pairing with the Y for dis-

junction on the Y-caused reduction in X exchange. The results below indicate that association between the Y and an autosome for disjunction occurs at the expense of the association between the Y and the X's at the time of exchange and, therefore, that nonhomologous pairing preceeds exchange. These observations are consistent with a single initiation of pairing as postulated by NOVITSKI (1964) but not with the two times of pairing separated by exchange proposed by GRELL (1962a).

It should be pointed out that the observation of nonhomologous pairing means only that nonhomologous chromosomes may pair, and does not necessarily mean a second kind of pairing. Evidence that these pairing events occur at different times in meiosis would be indicative of two types of pairing. The evidence presented in this report, however, are consistent with a single time of pairing in meiosis, and from this there is no reason to suppose two kinds of pairing. SANDLER and NOVITSKI (1956) postulate that chromosomes contain a region of general homology, shared by all chromosomes, as well as a region of specific homology, and that nonhomologous pairing occurs in the region of general homology. According to this view our designation of chromosomes as being either homologous or nonhomologous is misleading since it implies differences in pairing which might not exist.

Materials and Methods: All markers and chromosomes, with one exception as noted, are commonly available and are described by LINDSLEY and GRELL (1967). Standard culture techniques were employed. Details of specific crosses are given in the appropriate tables.

RESULTS

Analysis of In(1)w^{m4}: The results of measuring recombination in XX and XXY females homozygous for *In(1)w^{m4}* (*=In(1)3C1–2;20A*) are shown in Table 1,

TABLE 1

The results of crosses measuring secondary nondisjunction and crossing over (scored in sons) in females homozygous for In(1)w^{m4} *with and without an extra Y chromosome*

Cross No.*	Y used†	Non-crossovers	Crossovers, region							Matroclinous females	Patroclinous males
			1	2	3	1,2	1,3	2,3	1,2,3		
1	B^SY	630	150	171	...	10	...	...	...	59	64
	none	1411	416	395	...	35	...	...	...	4	17
2	B^SY	1953	386	572	...	30	...	...	...	148	109
	none	1680	421	526	...	36	...	...	...	1	3
3	sc^8Y	1809	410	525	...	46	...	...	...	123	109
	none	1691	507	553	...	40	...	...	...	4	4
4	Y	2054	462	601	...	41	...	...	...	107	101
	none	1385	360	370	...	42	...	...	...	0	6
5	B^SY	1021	239	399	372	26	58	30	1	90	59
	none	1220	420	401	372	41	85	30	1	1	0

* In crosses 1–4, the X-chromosome constitution of the maternal female was *In(1)w^{m4}, cv m f/In(1)w^{m4}*; in cross 5, the constitution was *In(1)w^{m4}, y cv m f/In(1)w^{m4}*. *In(1)w^{m4}* with *y* was kindly supplied by DR. R. F. GRELL. Region 1=*cv – m*, region 2=*m – f*, and region 3=*f –y*. Patroclinous males carried *B* and *y* or *w^a* to distinguish them from regular sons.

† The Y and non-Y-bearing females in each cross are sisters. XXY females in crosses 1, 2 and 5 are distinguished by having Bar eyes. In crosses 3 and 4 white eye mottling is suppressed in XXY females, resulting in red eyes.

TABLE 2

Comparison of map distances in females homozygous for In(1)w^{m4} *with and without an extra Y chromosome*

Cross No.	Y used	Percent nondisjunction*	Map distances for regions†			Total map distances
			1	2	3	
1	B^sY	10.9	14.8	16.7	...	31.5
	none		20.0	19.0	...	39.0
2	B^sY	9.1	13.0	18.8	...	31.8
	none		17.2	21.1	...	38.3
3	sc^sY	8.1	15.0	18.8	...	33.8
	none		19.6	21.2	...	40.8
4	Y	6.3	14.9	19.0	...	33.9
	none		18.6	19.1	...	37.7
5	B^sY	7.7	13.9	19.6	19.8	53.3
	none		21.3	18.4	19.0	58.7

* Percent nondisjunction = (twice matroclinous females/regular sons plus twice matroclinous females).
† Region 1 = *cv* — *m*, region 2 = *m* — *f*, region 3 = *f* — *y*.

and the map distances are given in Table 2. Crossing over appears to be normal in XX females but significantly reduced in XXY females, irrespective of the Y used (all exceptions carry nonrecombinant strands). (The term signifigence means that the samples compared are inhomogeneous by chi-square contingency tests.) The response by regions is not uniform, however, as the proximal region 1 always shows the greatest decrease, the middle region 2 is less affected, and in the one tested case the distal region 3 shows no change. The decrease in recombination appears correlated with the amount of nondisjunction. For example, cross 1 has the most nondisjunction and the largest relative decrease in recombination for regions 1 + 2 (approximately 19%), while cross 4 has the least nondisjunction and the smallest relative decrease in recombination in regions 1 + 2 (10%). Finally, in cross 5, with almost the entire arm marked, the addition of the Y can be seen to cause the frequency of no-exchange tetrads (E_0) to change from 7% to 13%, single-exchange tetrads (E_1) from 68% to 67%, and double-exchange tetrads (E_2) from 24% to 20%. (The two triple crossovers observed are treated as doubles in these analyses, otherwise making conventional assumptions.) Thus it appears that in *In(1)w*^{m4} homozygotes, in contrast to previously studied genotypes, the addition of a Y chromosome causes an increase in the incidence of E_0 tetrads.

Two models exist that predict an increase in E_0 tetrads with nondisjunction in XXY females. Each model postulates a frequency of XX bivalents with normal crossing over and a frequency of cells without XX bivalents with no crossing over so that recombination in XXY females = $P = B(1-A)$ where B is recombination in XX female controls and A is frequency of cells without XX bivalents. On BRIDGES' (1916) model of XY bivalent formation the univalent X goes to either pole one half of the time, generating exceptional gametes and noncrossover regular gametes in equal frequency; A is estimated by twice the frequency of non-

TABLE 3

A comparison of the observed recombination values for XXY females with the values expected on Bridges' (1916) bivalent-univalent model and Cooper's (1948) trivalent model, explained in text

		1		2		3		4		5	
Region	Model of	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
1	BRIDGES	15.6		14.1		16.4		16.3		18.0	
			14.8		13.0		15.0		14.9		13.9
	COOPER	17.8		15.6		18.0		17.4		19.7	
2	BRIDGES	14.9		17.3		17.8		16.7		15.6	
			16.7		18.8		18.8		19.0		19.6
	COOPER	16.9		19.2		19.5		17.9		17.0	
3	BRIDGES	...		...		...		...		16.1	
			...		...		...		...		19.8
	COOPER	...		...		...		...		17.5	
Total	BRIDGES	30.5		31.4		34.2		33.0		49.7	
			31.5		31.8		33.8		33.9		53.3
	COOPER	34.7		34.8		37.5		35.3		54.2	

disjunction. On COOPER's (1948) model, trivalent formation always yields an exceptional gamete and A = the frequency of nondisjunction. Both models predict a uniform decrease in recombination for all regions along the X chromosome. Although this is not the case with *In(1)w*^{m4}, the expectations on these models, based on control recombination values and nondisjunction for each cross, are compared with the observed values for XXY females in Table 3. It can be seen that few regions fit either expected value and that the data are not consistent with either the BRIDGES or COOPER models. Thus, because of the variation in regional responses, it seems likely that the origin of exceptional gametes with *In(1)w*^{m4} homozygotes is more complex than a single all or none alternative. The data are presented in this form to demonstrate an interesting, although not understood, consistency between the crosses. A pattern exists in that recombination is reduced more in region 1 than in region 2 for all crosses. This appears in Table 3 in the following way: the observed values for region 1 are less than either model predicts, while the observed values for region 2 are usually intermediate between the two expected values. However, when regions 1 and 2 are considered together, their sum closely approximates the bivalent-univalent model value for each cross. This doesn't imply the origin of exceptions on the bivalent-univalent model, since it doesn't hold for all three regions considered together, but it does imply a regular relation between nondisjunction and the decrease in recombination despite the variations in regional responses.

Analysis of inversions other than In(1)w^{m4}: The effect of the B^sY chromosome on recombination and disjunction of other inversion homozygotes is shown in Tables 4 and 5. Control crosses with normal chromosomes are included for com-

TABLE 4

Crossing over and nondisjunction in females homozygous for an X chromosome inversion with and without the $B^{s}Y$ *chromosome*

Chromosome tested	Y present	Non-crossovers	Crossovers, region*							Matroclinous females	Patroclinous males
			1	2	3	1,2	1,3	2,3	1,2,3		
+	yes	1298	608	324	...	36	...	...	...	97	97
	no	1360	552	311	...	45	...	...	...	1	2
In(1)dl 49	yes	2355	899	948	500	77	125	60	1	78	82
	no	1852	599	694	375	70	79	49	1	0	1
In(1)y^{4}	yes	3090	1490	1034	...	133	...	...	...	98	76
	no	2741	1149	904	...	119	...	...	...	2	7
In(1)sc^{4}	yes	2657	923	627	...	46	...	...	...	100	105
	no	2007	683	509	...	55	...	...	...	1	4
In(1)rst^{3}	yes	1115	378	348	...	46	...	...	...	46	45
	no	1168	489	369	...	58	...	...	...	1	0
In(1)B^{M1}	yes	1474	281	577	244	4	11	4	0	292	251
	no	1527	189	497	244	8	14	7	0	1	1
In(1)sc^{8}	yes	1109	438	290	...	38	...	...	...	52	41
	no	963	298	278	...	22	...	...	...	1	0
In(1)y^{3P}	yes	2055	650	502	...	39	...	...	...	120	109
	no	2207	563	568	...	36	...	...	...	3	17
In(1)sc^{8}	yes	1672	780	478	...	59	...	...	...	112	99
In(1)sc^{4}†	no	1311	347	312	...	51	...	...	...	1	1

* The markers in every cross are in coupling. For *In(1)dl 49*, region 1 = *sc* — *v*, region 2 = *v* — *g*, and region 3 = *g* — *f*. For *In(1)B*^{M1}, region 1 = *y* — *cv*, region 2 = *cv* — *v*, and region 3 = *v* — *g*. For all other crosses, region 1 = *cv* — *m*, and region 2 = *m* — *f*.

† Crossed to attached XY, *y B* males bearing *Dp(1;f)3* which carries the wild-type allele of *sc*.

TABLE 5

Comparison of map distances in females homozygous for an X-chromosome inversion with and without the $B^{s}Y$ *chromosome*

Chromosome tested	Y present	Percent nondisjunction	Map distances for regions			Total map distances
			1	2	3	
+	yes	7.9	26.2	14.6	...	40.8
	no		26.3	15.7	...	42.0
In(1)dl 49	yes	3.0	21.5	21.2	13.4	56.1
	no		20.1	21.9	13.6	55.6
In(1)y^{4}	yes	3.3	27.3	19.6	...	46.9
	no		25.8	20.8	...	46.6
In(1)sc^{4}	yes	4.5	21.8	15.1	...	36.9
	no		22.7	17.3	...	40.0
In(1)rst^{3}	yes	4.6	21.4	19.9	...	41.3
	no		26.2	20.5	...	46.7
In(1)B^{M1}	yes	18.4	9.3	18.4	8.1	35.8
	no		8.5	20.6	10.7	39.8
In(1)sc^{8}	yes	5.3	24.1	16.6	...	40.7
	no		20.5	19.2	...	39.7
In(1)y^{3P}	yes	6.9	19.8	15.5	...	35.3
	no		17.8	17.9	...	35.7
In(1)sc^{8}	yes	7.0	26.1	16.7	...	42.8
In(1)sc^{4}	no		19.7	18.0	...	37.7

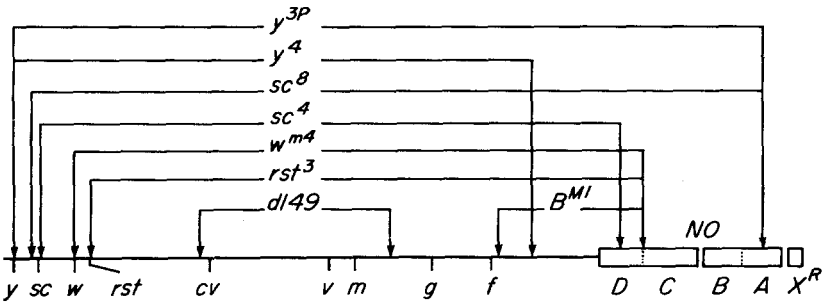

FIGURE 2.—Location of inversions on the genetic map and on COOPER's (1959) heterochromatic map.

parison. The inversions utilized are diagrammed in Figure 2. Sequences fall into two classes with respect to recombination: either addition of $B^{S}Y$ causes no change in the *cv-f* interval (which is the case for *In(1)dl49*, *In(1)y*^{4}, *In(1)sc*^{8} and *In(1)y*^{3P}, as well as normal chromosomes) or a 7–20% reduction in the *cv-f* interval (which is the case for *In(1)rst*^{3}, *In(1)sc*^{4} and *In(1)B*^{M1}). Those sequences with reduced crossing over all have their right break point in the distal half of the proximal heterochromatin.

Also included in Tables 4 and 5 are the results of a cross with females heterozygous simultaneously for *In(1)sc*^{8} and *In(1)sc*^{4}. This was designed to test the effect of heterozygosity for a break in the distal half of the proximal heterochromatin on whether the response in chromosome behavior in females to a Y is normal, like *w*^{m4}-homozygotes, or is perhaps intermediate. The results show that such a combination is normal in its behavior. Thus the normal behavior of chromosomes may be said to be dominant to the behavior of *w*^{m4}-type chromosomes. Similarly $+/In(1)w^{m4}/Y$ and $+/In(1)w^{m4}$ females show equal recombination. If this inference is generally valid then the absence of an appreciable effect of the Y chromosome on recombination in previously studied genotypes (GRELL 1962b) is consistent with these observations since all carried at least one normal chromosome.

TABLE 6

A comparison of the observed recombination values for XXY females homozygous for an X-chromosome inversion with the values expected on Bridges' (1916) bivalent-univalent model, explained in text

Chromosome tested		Regions 1	2	3	Total
In(1)sc^{4}	Observed	21.8	15.1	. . .	36.9
	Expected	20.7	15.7	. . .	36.4
In(1)rst^{3}	Observed	21.4	19.9	. . .	41.3
	Expected	23.8	18.6	. . .	42.4
In(1)B^{M1}	Observed	9.3	18.4	8.1	35.8
	Expected	5.4	13.0	6.8	25.2

A comparison is made in Table 6 between the observed recombination values for XXY females and the values expected on the bivalent-univalent model of BRIDGES for the *In(1)sc*^{4}, *In(1)rst*^{3} and *In(1)B*^{M1} crosses. The cases of *In(1)sc*^{4} and *In(1)rst*^{3} are similar to *In(1)w*^{m4} in that the bivalent-univalent model provides a good fit for the combined *cv-m* and *m-f* intervals although the regions considered individually do not fit the model. This suggests that whatever the relation between nondisjunction and reduction in exchange in *In(1)w*^{m4}, the same relation also applies to *In(1)sc*^{4} and *In(1)rst*^{3}. In *In(1)rst*^{3}, like *In(1)w*^{m4}, recombination in XXY females between *cv* and *m* is decreased more than is recombination between *m* and *f*; whereas in *In(1)sc*^{4}, recombination in the *m-f* region is reduced more than that in the *cv-m* region by the presence of a Y. This is shown in Table 6 for *sc*^{4} where it may be seen that the expected value for the *m-f* interval is larger than observed and the expected value for the *cv-m* interval is smaller than observed. The *sc*^{4} data are pooled from two experiments which show a similar response to the addition of a Y chromosome. It might be inferred from the *w*^{m4} or *rst*^{3} data, where the proximal region is most affected by the Y, that this inhibition is the result of X-Y competitive pairing which starts at the centromere end and spreads along the X for varying lengths. However, this notion cannot explain the *sc*^{4} data, which requires competitive pairing to start at the tip and spread towards the centromere. Thus the decrease in exchange in XXY females appears to be more complex than hitherto suspected on the basis of X-Y competitive pairing.

The case of *In(1)B*^{M1} in Table 6 differs from *In(1)w*^{m4} in that the observed recombination values are larger than the values expected on the bivalent-univalent model for each region. The direction of this discrepancy for the measured regions could be a consequence of an absence of exchange in the inverted region in XXY females. This possibility is suggested by the observation (GRELL 1962b) that females heterozygous for *In(1)B*^{M1}, where exchange between *f* and the centromere should be virtually absent, yield the same frequency of secondary nondisjunction as do the homozygotes (Table 5).

Thus, there are exceptions to the generalization that exchange is always equal in XX and XXY females, notably homozygotes for inversions whose right break point is distal to the nucleolus organizer in the proximal heterochromatin. The differences in recombination described between XX and XXY sisters homozygous for these inversions are approximately an order of magnitude larger than the minor differences between *f* and the centromere noted by GRELL (1962b) and STURTEVANT and BEADLE (1936), which are without effect on X-nondisjunction, and they suggest that homozygosity for a break in a part of the basal heterochromatin in some way damages the chromosome, with the result that the Y can interfere with X-exchange. In a sense this damage makes possible the inference of X-Y association at exchange, which presumably occurs but is usually not seen with normal X-chromosomes. In these homozygotes the frequency of no-exchange tetrads is increased in XXY females which, on occasion, could lead to frequencies of secondary nondisjunction, like that obtained by OKSALA (1958), which are 2 to 3 times higher than those observed with normal chromosomes. This result

does not, by itself, provide information on the time of nonhomologous pairing because it may be argued that the Y chromosome shares conventional homology with the X chromosomes which under certain conditions interferes with exchange in the X chromosomes. Finally, a highly regular relation between the decrease in recombination in XXY females and secondary nondisjunction in those females is inferred from the consistency with which the bivalent-univalent model of BRIDGES fits the values of *cv-f* recombination for the w^{m4}, rst^{3} and sc^{4} chromosomes.

Analysis of the Y effect on exchange in females heterozygous for both In(2LR)SM1 *and* T(2;3)A: It is generally agreed that in females of the constitution *T(2;3)A/In(2LR)SM1*; +, disjunction of the translocation and the normal third chromosome is directed by exchange between chromosome 3 and its homologous regions in the two arms of the translocation, whereas *In(2LR)SM1* is not often involved in exchange and is generally free to disjoin from another element. *In(2LR)SM1* [= *In(2LR)22A3–B1;60B–C* superimposed on *(In(2L)-22D1–2;33F5–34A1* + *In(2R)42A2–3; 58A4–B1*] and *T(2;3)A* [= *T(2;3)-39B–C;83B*]. In $In(1)w^{m4}/In(1)w^{m4}$Y; *T(2;3)A/In(2LR)SM1*; + females, the Y can be shown to segregate frequently from *In(2LR)SM1* (hereafter abbreviated *SM1*) and to promote considerably less secondary nondisjunction than in a normal autosomal genotype (OKSALA 1958). The inferred pairing relations are diagrammed in Figure 3. The advantage of using a translocation in addition to *SM1* is that it allows a direct demonstration of Y-*SM1* segregation which can only be inferred from females heterozygous for *SM1* alone, since in the latter case the normal 2 can also disjoin from the Y such that there is no apparent segregation of Y and *SM1*. The effect of the Y chromosome on exchange in $In(1)w^{m4}$ homozygotes should allow us to distinguish whether the association between *SM1* and Y leading ultimately to their segregation occurs after exchange as proposed by GRELL or prior to exchange as suggested by NOVITSKI. Association prior to ex-

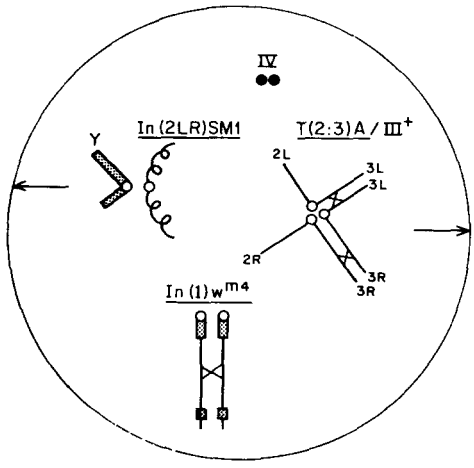

FIGURE 3.—Diagram of the inferred pairing relations in $In(1)w^{m4}/In(1)w^{m4}$/Y; *T(2;3)A/In(2LR)SM1*;+ females.

TABLE 7

Results from crosses of sisters of the constitution In(1)w^{m4}, cv m f / In(1)w^{m4} *with and without the* B^{s}*Y chromosome and with and without* In(2LR)SM1 *and* T(2;3)A *(marked with* Bl*) to* y B *males*

Autosomal constitution	Y present?	Progeny* B^s females			non-Y females			w^{m4} females			y B males			w^{m4} ♂♂, crossovers by regions			
		SM1	Bl	+	*SM1*	Bl	+	*SM1*	Bl	+	*SM1*	Bl	+	zero	1	2	1+2
+/+; +/+	yes	...	...	1310	...	...	1792	...	...	135	...	...	117	2232	429	665	48
	no	...	...	...	...	...	3735	...	...	2	...	...	9	2450	577	714	61
T(2;3)A/+;+	yes	...	244	380	...	518	550	...	21	27	...	33	20	1118	224	357	25
	no	...	...	...	...	856	1014	...	...	1	...	...	1	1056	283	375	27
SM1/+; +/+	yes	322	...	293	416	...	522	16	...	18	17	...	23	1069	306	377	42
	no	...	...	...	1485	...	1495	1	...	1	4	...	4	1803	682	606	98
T(2;3)A/SM1;+	yes	53	352	...	873	100	...	5	9	...	13	9	...	899	250	323	46
	no	...	...	...	850	712	...	...	5	..	4	1	...	863	278	282	46

* B^s ♀♀ and non-Y ♀♀ are regular daughters; w^{m4}♀♀ are exceptional matroclinous daughters and *y B* ♂♂ are exceptional patroclinous sons. w^{m4} ♂♂ are regular sons, scored for crossing over: zero = noncrossover sons 1, 2, and 1+2 are sons with crossovers in the *cv — m*, *m — f* and both intervals respectively.

TABLE 8

Comparison of map distances for the cv-m *and* m-f *intervals in females homozygous for* In(1)w^{m4} *with and without* B^{s}*Y*, In(2LR)SM1, *and* T(2;3)A

Autosomal constitution	Y present?	Percent non-disjunction	Map distances for regions 1	2	Total map distances
+/+; +/+	yes	7.4	13.1	19.6	32.7
	no		16.8	20.4	37.2
T(2;3)A/+;+	yes	5.3	13.7	21.0	34.7
	no		17.8	23.1	40.9
SM1/+;+/+	yes	3.7	18.7	22.5	41.2
	no		23.2	22.4	45.6
T(2;3)A/SM1;+	yes	1.8	19.2	24.0	43.2
	no		22.0	22.3	44.3

change at the expense of X-Y association should result in a decrease of the Y chromosome's effects on both recombination and nondisjunction; whereas Y-*SM1* association following exchange should affect the frequency of nondisjunction but not of recombination. It is recognized that this test attempts to demonstrate an exchange event that is not independent of nonhomologous pairing; failure to do this would only continue the generalization that exchange is independent of nonhomologous pairing which is consistent with both the GRELL and NOVITSKI sequences of meiosis.

The results of such a test measuring recombinaiton (in sons), X nondisjunction, and segregation of marked autosomes with B^sY (in daughters) in XX and XXB^sY females homozygous for *In(1)*w^{m4}, with and without *In(2LR)SM1* and *T(2;3)A* are given in Table 7 and their map distances in Table 8. It is clear from these data that the difference in X-recombination between XX and XXY sisters with the same autosomal genotype decreases with competition of the autosomes for association with the Y chromosome. The relative index [*Recombination in XXY* ♀♀*/Recombination in XX* ♀♀] increases from .88 in females with normal autosomes to .97 in females heterozygous for both *SM1* and *T(2;3)A*. The amount of nondisjunction is correlated with the decrease in *cv-f* crossing over in XXY females for each autosomal genotype: the map values expected on the relation $P = B(1-A)$, where B is recombination in XX sisters and A is twice secondary nondisjunction, are plotted against the observed map values for XXY females in Figure 4 for these data and for the other w^{m4}-, sc^4-, and rst^3-crosses. (The observed map values for XX females are included for comparison.) It can be seen that these points describe a straight line, that the points for the genotypes *SM1*+; +/+ and *T(2;3)A/SM1*;+ fall on the line, and therefore that the relation between secondary nondisjunction and the decrease in recombination is retained in *SM1*/+; +/+ and *T(2;3)A/SM1*;+ females. Thus, manipulating the degree of secondary nondisjunction by introducing *SM1* as an alternate pairing partner for the Y also changes the Y's effect on X-exchange and by the same amount. Moreover, the amount of Y-*SM1* nonhomologous association in these XXY; *T(2;3)A/SM1*; + females is 75% which corresponds almost exactly to the reduction in effect of the Y on both recombination (from 12% to 3% difference between XX and XXY sisters) and disjunction (7.4% to 1.8% secondary disjunction) caused by supplying *SM1* as an alternative pairing partner for the Y.

These results are presented in a different form in Figure 5 as are the results of the other *In(1)*w^{m4} crosses; the increase in percent tetrads with no exchange between *cv* and *f* is plotted against secondary nondisjunction for each cross. The correlation between nondisjunction and the effect of the Y on exchange is clearly evident in this figure, as is the effect of *SM1* and *T(2;3)A* in reducing the difference in exchange between XX and XXY sisters. Thus, exchange in XXY females becomes more like exchange in their XX sisters with decreasing nondisjunction.

It is possible, though unlikely, that some hitherto unsuspected property of the interchromosomal effect of *SM1* increasing X-exchange is responsible for the change in the effect of the Y on X-recombination. (It should be perhaps restated that recombination is compared in XX and XXY females only between those

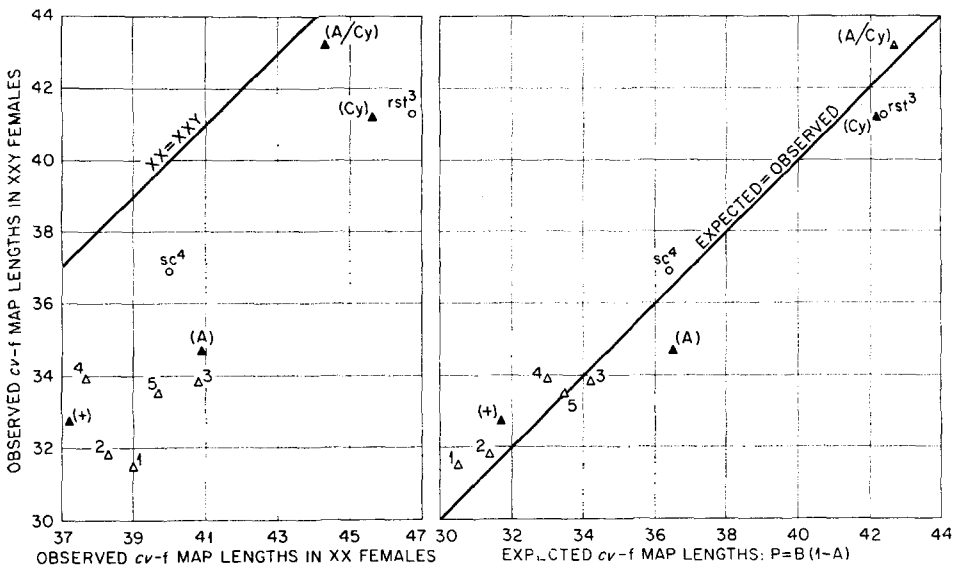

FIGURE 4.—A comparison of map values for XX and XXY females with the map values for XX females and the values expected on the relation $P = B(1 - A)$, explained in text; the numbered points (open) refer to crosses in Table 1, lettered points (closed) refer to crosses in Table 7: + = normal autosomes, $A = T(2;3)A/+;+$, $Cy = In(2LR)SM1/+$; $+/+$, $A/Cy = T(2;3)A/In(2LR)SM1;+$, the open circles refer to the crosses in Table 5, and the lines designated XX = XXY and Expected = Observed mark the same values for ordinate and abcissa.

sisters with the same autosomal genotype. Therefore the decrease noted in effect of the Y on recombination is not caused by the interchromosomal effect *per se.*) For this to be the case it would be necessary to assume that the Y effect on exchange is itself an interchromosomal effect decreasing X-exchange to which the

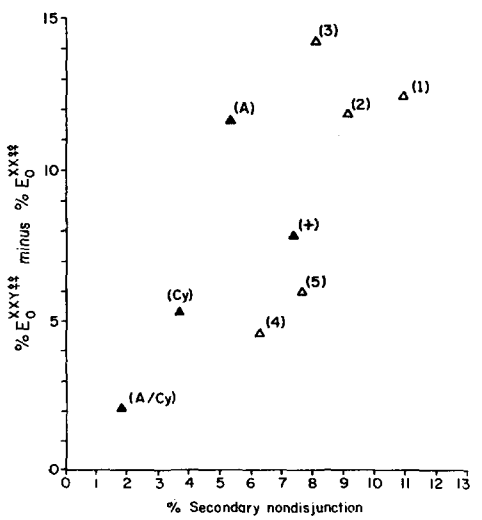

FIGURE 5.—The increase in percent no-exchange tetrads from XX to XXY females homozygous for $In(1)w^{m4}$ plotted with secondary nondisjunction; the numbered points (open) refer to crosses in Table 1, lettered points (closed) refer to crosses in Table 7: + = normal, autosomes, $A = T(2;3)A/+;+$, Cy = $In(2LR)SM1/+$; $+/+$, and $A/Cy = T(2;3)A/In(2LR)SM1;+$.

SM1 interchromosomal effect is dominant. The results do not support the notions that the difference in recombination between XX and XXY sisters decreases with increasing map length, or that there is somehow more *SM1* interchromosomal effect in XXY than in XX females. On the former notion it can be seen in Figure 4 where map values for XX and XXY sisters are compared that, if anything, the difference in recombination would be expected to increase with higher recombination in XX females. On the latter notion it can be seen in Table 8 that recombination and, hence, the interchromosomal effect are equal in XX; *SM1*/+; +/+ and XX; *T(2;3)A/SM1*;+ females but recombination in XXY; *SM1*/+; +/+ females is lower than that in XXY; *T(2;3)A/SM1*;+ females. The difference in recombination between XX and XXY sisters of the genotype *SM1*/+; +/+ is significant whereas the difference between XX and XXY females of the genotype *T(2;3)A/SM1*;+ is not significant. Therefore the "extra" interchromosomal effect in XXY; *SM1* females does not seem to be sufficient to account for the observed decrease in the Y effect on recombination.

It is perhaps surprising that recombination and secondary nondisjunction are not equal in *T(2;3)A/SM1*; + and *SM1*/+; +/+ XXY females, since on the hypothesis of Y-*SM1* pairing before exchange such an event might be expected to occur with equal probability in females of those two genotypes. However, the observation that the probability of *SM1* pairing nonspecifically with its homologue or with the Y differs between these two autosomal genotypes could not be known before the experiment was performed, although it is reasonable since with *SM1*+; +/+ females there are three elements to be considered (i.e., *SM1*, the normal 2, and B^sY) whereas with *T(2;3)A/SM1*; + females there are either five or two elements to be considered (i.e., *SM1*, 2L+3L, 2R+3R, the normal 3, and B^sY, or just *SM1* and B^sY). On the other hand, the difference in recombination between XXY; *SM1*/+; +/+ and XXY; *T(2;3)A/SM1*; + females is correlated with nondisjunction, and thus the positive conclusion can be drawn that Y-*SM1* nonhomologous pairing frees the X's to pair and undergo exchange like their XX sisters. Since *SM1* and Y are recovered independently among exceptions which results from X-Y association, these results are in agreement, quantitatively as well as qualitatively, with the NOVITSKI hypothesis that the association in which the X's and *SM1* compete for the Y as a disjunction partner occurs before exchange.

DISCUSSION

This report is concerned with determining whether the initiation of nonhomologous pairing occurs at the same time or after the initiation of regular homologous pairing. The results above indicate that a necessarily disjunctional pairing event can be demonstrated to influence exchange, which is consistent only with the view that the initiation of nonhomologous pairing occurs before exchange. Thus the initiation of pairing for both exchange and disjunction proceed exchange and it is therefore sufficient to postulate a single meiotic association. What follows is a hypothetical series of events in meiosis which is consistent with all of the observations to date. (1) Chromosomes associate, or are associated, in a chromocenter;

the chromocenter may already exist as a consequence of the preceeding mitotic anaphase polarization. In any case it fulfills the need for long range specific pairing forces previously thought necessary to bring elements together. (2) Holologous chromosomes find each other in the chromocenter and synapse. ROBERTS (1966) hypothesizes that this event regularly occurs in two steps: an alignment of homologous arms so that homologous regions are brought into proximity throughout the chromosome (= "recomplex" formation), followed by the more intimate base-by-base pairing associated with recombination. Synapsis of nonhomologous chromosomes in the regions of general homology can occur either during this event or earlier in the chromocenter, although it is perhaps more unifying to consider this an example of the alignment bringing homologous regions into proximity as described above. It is inferred from the results of COOPER (1948) that in XXY females an arm of the Y can pair with only one X chromosome; therefore pairing of nonhomologous chromosomes, as well as homologous chromosomes, seems to be by two's. However, multivalent formation generally or trivalent formation, which certainly occurs, is possible by means of two-armed elements that pair with a different element for each arm. The best known example of this is that formed by X-Y-X, with one X on each Y arm. (3) Exchange orients tetrads such that recombinant chromosomes almost always disjoin regardless of how they may be paired in the regions of general homology. Thus, exchange sorts out whatever trivalents have occurred earlier. It is only among no-exchange tetrads that the association of nonhomologous chromosomes is expressed in preferential disjunction of those nonhomologues. This gives the appearance of homologous pairing being stronger, or occurring earlier, than nonhomologous pairing.

It is interesting to note that in $+/In(1)w^{m4}/Y$; *T(2;3)A/SM1*; + females the pair of X's heterozygous for the inversion competes more efficiently for the Y than the pair homozygous for $In(1)w^{m4}$. The segregation classes of B^sY, *SM1* and *T(2;3)A* among the progeny produced by sisters of both genotypes are compared in Table 9. Heterozygosity for $In(1)w^{m4}$ decreases B^sY-*SM1* nonhomologous pairing from 75% to 57% while increasing secondary nondisjunction from 2.6% to 6.1%. The increase in secondary nondisjunction, although a change in the right direction, does not fully account for the decrease in B^sY-*SM1* nonhomologous pairing. Unless the interchromosomal effect of $+/In(1)w^{m4}$ markedly increases exchange between *SM1* and *T(2;3)A* this difference is unexpected on the distributive pairing hypothesis of GRELL because in a distributive pool consisting only of noncrossover X's, *SM1* and B^sY, a change in the frequency of X-Y pairing should correspond closely to a change in Y-*SM1* pairing. It is understandable on the NOVITSKI thesis of pre-exchange nonhomologous pairing because the real increase in X-Y associations is only partially expressed since most X tetrads undergo exchange. It is as yet unknown whether the increase in X-Y association observed with females heterozygous for $In(1)w^{m4}$ is due to the presence of an uninterrupted heterochromatic region or to a consequence of mechanical difficulty in chromosome-level pairing with the heterozygous inversion, perhap by slowing down "recomplex" formation. Thus it might seem that pairing in areas of specific

TABLE 9

Segregation of B^sY *with* In(2LR)SM1 *and* T(2;3)A *in XXY females homozygous or heterozygous for* $In(1)w^{m4}$, *and X chromosome nondisjunction*

Progeny receiving	Mother's genotype	
	homozygous w^{m4}	heterozygous w^{m4}
$B^sY + SM1$	66	37
$B^sY + T(2;3)A$	351	79
non-Y, *SM1*	878	255
non-Y, *T(2;3)A*	109	55
Total gametes	1404	426
Exceptional gametes	36	26*
% X nondisjunction	2.6	6.1
n†	12.5	21.6
a	75.0	56.8

* Exceptional gametes = twice matroclinous females.
† n = percent nondisjunction of B^sY and *SM1*. $a = 1 - 2n$.
= the frequency of *SM1* — Y nonhomologous pairing (GRELL and GRELL 1960).

homology can influence the pairing of generally homologous regions, but not *vice versa.*

It is implicit in this hypothetical series of events that pairing in the regions of general homology does not normally influence pairing in the regions of specific homology, and does not itself result in recombination. What regions of the chromosome possess these characteristics? Some recent results of R. F. GRELL (1967) are of significance on this question. She finds that duplications containing X-euchromatin as well as heterochromatin suppress crossing over in the duplicated region of the normal chromosomes. However, in a system of XXDp females homozygous for *T(3;4)86D* with a free fourth chromosome (= triplo-IV females) in which the univalent 4 competes with the X's for disjunctive pairing with the duplication, she finds that the inhibitory effect of the duplication on exchange is unaffected. Thus, exchange in X's and nonhomologous pairing of *Dp* and 4 are independent, from which she concludes they must be sequential. However, this need not contradict the conclusion drawn above with $In(1)w^{m4}$ since it is possible that a duplication containing a region of specific homology as well as a region of general homology can pair simultaneously and independently for both regions. For example, that describes the behavior of regular chromosomes. Moreover, since the observation that strictly heterochromatic duplications (e.g. the Y chromosome) but not duplications containing both euchromatin and heterochromatin, can be withdrawn by nonhomologous pairing from affecting exchange, strongly suggests that the general regions of homology are heterochromatic, as proposed by SANDLER and NOVITSKI (1956). NOVITSKI and BRAVER (1954) were the first to make this suggestion with respect to how heterochromatin behaves; they placed the time of heterochromatic pairing prior to that of euchromatic pairing, although by the nature of their data they were concerned with pairing within rather than between chromosomes.

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SUMMARY

The generalization that exchange is always equal in XX and XXY females is not true for females homozygous for those inversions whose right break point is distal to the nucleolus organizer in the proximal heterochromatin. In such genotypes the frequency of no-exchange tetrads is increased by the presence of an extra Y chromosome, thus marking the position of the Y at the time of exchange. This situation allows us to determine whether the initiation of pairing of nonhomologous chromosomes for disjunction occurs before or after exchange. Providing *In(2LR)SM1* as an alternative pairing partner for the Y allows exchange in XXY females to increase, reaching the level of their XX sisters The data provide a direct demonstration that a necessarily disjunctional pairing event influences exchange, which is consistent only with the view that the initiation of nonhomologous pairing preceeds exchange, as postulated by NOVITSKI (1964). Thus they are consistent with a single time of chromosome pairing in meiosis and therefore do not require two kinds of pairing. A hypothetical series of events in meiosis which is consistent with all observations to date is included.

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