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A New Hypothesis Conerning the Sequence and Nature of Meiotic Events in the Female of Drosophila Melanogaster

Rhonda F. Grell
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To the Graduate Council:

I am submitting herewith a dissertation written by Rhonda F. Grell entitled "A New Hypothesis Conerning the Sequence and Nature of Meiotic Events in the Female of Drosophila Melanogaster." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Dr. J. Gordon Carlson, Major Professor

We have read this dissertation and recommend its acceptance:

Dr. Dan L. Lindsley, Dr. E.H. Grell, Dr. T. Salo, Dr. Drew Schwartz, & Dr. Brook Webber

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

December 1, 1961

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Gordon Carlson
Major Professor

We have read this dissertation
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Ronald C. Fraser

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Dean of the Graduate School

A NEW HYPOTHESIS CONCERNING THE SEQUENCE AND NATURE OF MEIOTIC EVENTS IN THE FEMALE OF *DROSOPHILA MELANOGASTER*

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Rhoda F. Grell

December 1961

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R. F. G.

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I. INTRODUCTION

Three of the basic phenomena comprising the meiotic process are synapsis, crossingover and disjunction. It is generally conceded that some form of pairing must precede crossingover; that the crossingover process occurs prior to disjunction; and that disjunction implies a previous association of elements. Construction of a model describing the causal relations among the three events might be expected to be fairly simple, since the possibilities are limited. Yet, no model has been proposed that is consistent with all the genetic data.

Like many processes, meiosis is more susceptible to analysis under abnormal conditions that interfere with one or more of the events concerned. Thus, Bridges[1] showed that the presence of an extra Y chromosome in the female, significantly increases the frequency of nondisjunction of the X's. Since secondary exceptions, i.e., individuals arising from XX-Y segregations in a Y-bearing mother, in contrast to regulars, are noncrossovers, this result was interpreted to mean that the difference between the two types was initiated at a stage preceding crossingover and that the exceptions arose from those cases in which XY instead of XX synapsis occurred.

Anderson[2] found that a heterozygous X-autosomal translocation in XXY females resulted in a high frequency of X-nondisjunction. In spite of a marked reduction of crossovers in the exceptional classes, the percentage of X-chromosome crossovers among the combined regular

and exceptional progeny was apparently the same as that found in the progeny from XX mothers. Anderson concluded that synapsis and crossing-over between the X's was not affected by the presence of a Y chromosome but, after that crossing over had taken place, the Y could cause "the more loosely paired X chromosomes to be distributed to the same pole." The interpretations of Bridges and of Anderson as to the role of the Y chromosome in synapsis, exchange and disjunction of the X's are clearly incompatible.

Dobzhansky,[3] on the basis of studies concerning the effects of translocations on the crossingover and disjunctive processes, formulated the hypothesis of "competitive pairing." According to this view, crossingover and nondisjunction are negatively correlated. The relationship, however, is not a direct causal one in the sense that crossingover inevitably leads to regular disjunction and failure of crossingover inevitably leads to random assortment. Rather, Dobzhansky assumed that both processes are predetermined by the intimacy of synapsis between specific, homologous loci prior to crossing over. Rearrangements (translocations, inversions, duplications) that provoke a conflict between the attraction forces, weaken the intimacy of synapsis between homologs and lead to both decreased crossingover and to increased nondisjunction.

Sturtevant and Beadle's[4] studies with heterozygous X-inversions verified that exchange is not a prerequisite for normal disjunction in female Drosophila, since from In (1) delta-49 heterozygates no exceptional females, i.e., primary exceptions arising from XX-0 segregation in an XX mother, were recovered from among some 3,000 daughters in spite

of the fact that an estimated half of the tetrads were noncrossovers. The possibility that a high rate of exchange, undetected by Sturtevant and Beadle, occurred in the proximal heterochromatin, was effectively ruled out by Cooper.[5] The increase in secondary exceptions, occasioned by the presence of the heterozygous X-inversions, was found to show a high positive correlation with the increase in noncrossover tetrads. This indicated to Sturtevant and Beadle that secondary nondisjunction was primarily dependent on the occurrence of noncrossover tetrads whereas they considered that the Y reduced crossingover proximally in the X's in accordance with the hypothesis of competitive pairing.

No one of the models postulated adequately explains the genetic data relating to secondary nondisjunction. If pairing is competitive and occurs only between specific homologous loci, the effect of the Y on crossingover should be localized in the proximal region where the X and Y share homology. Distal regions should show crossover values approaching normal, both in the regular and exceptional progeny. Yet, exceptions are noncrossovers. Bridge's hypothesis, that XY pairing eliminates XX exchange, requires that a reduction in crossingover, paralleling the amount of secondary nondisjunction, occur throughout the X chromosomes. Sturtevant and Beadle's evidence, as well as Anderson's, indicate that the presence of a Y does not appreciably reduce crossingover between the X's, even when the frequency of exceptions is high. Finally, Anderson's interpretation, that the Y acts only after crossing over is completed, does not account for the localized, yet consistent, proximal reduction in crossingover.

The discovery that nonhomologous elements are capable of very high frequencies of association, as inferred from their segregation behavior,[6,7,8] suggests a new method of attack upon these problems. In contrast to the XXY situation, the relation of the extra element to the pair of chromosomes under study is uncomplicated by homology. A priori, the occurrence of nonhomologous associations is in itself puzzling, for the precise, highly specific pairing required for exchange seems incompatable with nonspecific association. Dobzhansky[9] recognized that nonspecific attractions between chromosomes do exist as evidenced by McClintock[10] and Burnhams[11] observations of nonhomologous associations of prophase chromosomes in maize. He did not attempt to reconcile the concept of nonspecific pairing with the highly restrictive type of pairing upon which his "competitive" hypothesis was predicated. He does consider that nonhomologous association "preclude the occurrence of normal crossingover."

The present work is divided into two general classes of experiments. In the first, the relation between exchange and secondary nondisjunction in the X chromosomes is reexamined. Previous studies have utilized situations where crossingover is high but nondisjunction extremely low (i.e., +/+/Y) or where crossing over is low and nondisjunction high (i.e., +/dl-49/Y). These experiments examine the relation between exchange and disjunction in inversion heterozygotes with intermediate levels of recombination and nondisjunction, so that a sizeable increase in nondisjunction, in the presence of a Y chromosome, could be correlated with a sizeable decrease in crossing over, if such were the

case. In the second series of experiments, a situation is created in which chromosome two exhibits a high incidence of nonhomologous association with the Y chromosome; the relation between exchange in chromosome two and Y-2 association is examined.

The results indicate that the meiotic process in the female of Drosophila is separable into two phases, that concerned with crossing-over and that concerned with disjunction. They further indicate that the Y chromosome is slightly involved in the first phase, due to limited homology with the X, but that it is highly involved in the second. In order to account for these relationships, two kinds of pairing have been postulated, one preceding exchange and a second preceding disjunction.

II. METHODS AND MATERIALS

The rate of crossingover is known to be readily altered by the genetic background, the temperature and the age of the female. The present experiments were planned so that the test (XXY) females and the control (XX) females were sisters, thus insuring uniformity of genetic background for the two groups. Virgin females, whose age did not exceed 10 hours, were placed, singly, in vials with three males for 24 hours. The temperature was maintained at 25° ± 1° C. for this period. Flies were then transferred to bottles for a six day period. The flies were kept at 25° ± 1° C. until eclosion began.

Secondary Nondisjunction

Crossingover and disjunction of the X chromosome were studied in normal and in extra Y females in the absence and presence of heterozygous X-inversions. Two inversions, In (1) Bar^{MI}(In (I) B^{MI})^{12} and In (1) $scute^{7}$ (In (I) sc^{7})^{12} were employed. The former, which carries the inseparable dominant marker Bar^{MI} ($\underline{B}^{MI}$) (whose phenotype is a weak bar eye clearly distinguishable from the more extreme bar eye of Bar^{I} ($\underline{B}^{I}$)), is located in the most proximal euchromatic region of X. It includes about one-sixth of the total length of the salivary gland X-chromosome. In (I) sc^{7} is distally located and includes a little less than one-fourth of the total length. Heterozygous recessive markers were introduced into the uninverted regions of the chromosomes and into their homologs at appropriate intervals in order to

measure exchange. Only the male progeny were scored for crossovers.

Crossingover was measured for the entire length of the uninverted X-chromosome, including the region between the most proximal marker, carnation (car), and the tip of the right arm. This was accomplished by the use of Inp (I) scute^{VI,13} a pericentric inversion of the X chromosome that places y^+ to the right of the centromere. The entire left arm of sc^{VI}, to a point beyond the forked (f) locus, was replaced with an equivalent portion of the X carrying y^2, sc, v and f so that the chromosome now carries a duplication for the yellow locus. Again, only male progeny were scored for crossovers.

Disjunction of the X chromosomes was followed by the presence of the mutants y and B in the tester males. The phenotype of the exceptional males was y and B whereas regular males, with respect to these mutants, were y^2 or y^+ and B^{MI} or B^+. In the cases of the inversion heterozygotes the tester males carried an attached XY^{14} and a free Canton-S Y chromosome. The XY was used to increase the viability of primary male exceptions.

The extra Y in the females was an unmarked, Canton-S Y so that classification of the mothers as to the presence of a Y could not be made until the progeny were observed. This was done for the two inversion heterozygotes, In (I) $B^{MI}/+$ and IN (I) $sc^7/+$, by noting the frequency of the exceptional progeny. Secondary exceptions constitute about 11 per cent from B^{MI} heterozygotes and about 12 per cent from sc^7 heterozygotes. Primary exceptions number less than 1 per cent in each case.

The low frequency of secondary exceptions in the case of the uninverted X-chromosomes does not permit a reliable distinction to be made between the XXY and XX mothers. A different method for identifying the presence of an extra Y in these females was devised. This utilizes in the tester male, an X chromosome carrying a rearrangement of the bar locus called Bar^{SV6} ($\underline{B}^{SV6}$).[15] The bar phenotype now displays a variegated position effect that ranges from a weak to an intermediate bar. In the presence of an extra Y the expression of $\underline{B}^{SV6}$ is much more extreme. From a mating of XXY females to males carrying $\underline{B}^{SV6}$, two approximately equal classes of regular daughters are distinguishable; those with very narrow bar eyes (X/X, $\underline{B}^{SV6}$/Y) and those with weak to medium (rarely extreme) bar eyes (X/X, $\underline{B}^{SV6}$). A mating of XX females to $\underline{B}^{SV6}$ males produces the latter class exclusively. This method is completely reliable for identifying the presence of an extra Y chromosome in a parent.

Nonhomologous Association

Association between nonhomologues at meiosis is assumed to occur when two or more chromosomes are present without adequate homologous pairing partners.[16] It is recognized by the nonrandom assortment of the nonhomologues. In the present experiments, nonhomologous association between the Y chromosome and chromosome two has been studied. Chromosome two was rendered inadequate for normal pairing by the presence of multiple inversions. Its homolog, through involvement in a translocation, was frequently eliminated as a pairing partner, permitting

the inverted chromosome, in these instances, to associate with the Y.

The inverted chromosome that was used, Ins (2 LR) Gla,[12] carries the inseparable dominant marked, Glazed (Gla). The size of the inverted segments insures adequate room for the measurement of crossingover distal to the breakpoint in 2R and heterozygous recessive markers were introduced into this region for that purpose. As described in Bridges and Brehme,[12] In (2 LR) Gla is a single pericentric inversion with one break at 27F and the second at 51D and should permit crossing over in 2L as well as 2R. Failure to recover any crossovers distal to the 2L inversion breakpoint among some 12,000 flies prompted a salivary gland chromosome analysis which disclosed the presence of two additional breaks, probably identical to those present in inversion (2L) Cy, at 22D and 33F. It appears likely that the Glazed inversion was originally induced in In (2L) Cy.

The two translocations used, T (2;3) A^{12} which carries the inseparable dominant marker Bristle (Bl) and T (2;3) 101,[12] are both reciprocal 2;3 translocations with breaks very close to the spindle attachments. T (2;3) A gives the rearrangement 2L + 3L and 2R + 3R whereas T (2;3) 101 gives the rearrangement 2L + 3R and 2R + 3L. The presence of the inseparable, easily classifiable and fully penetrant dominant marker, Gla, in the inversion provided a means for following the segregation of the second chromosomes. Use of a marked sc^8 Y^{17} that carries the normal allele of yellow (y^+) and the introduction of $yellow^2$ (y^2) into the X chromosomes of both parents provided the means for following the distribution of the Y chromosome among the progeny.

The control females consisted of sisters of the test females which, except for the absence of the Y chromosome, were of the same genetic constitution.

The males in all cases were $\underline{y}^2$; $\underline{al}$ $\underline{px}$ $\underline{sp}$/$\underline{al}$ $\underline{px}$ $\underline{sp}$.

III. RESULTS AND ANALYSIS

Secondary Nondisjunction

The extent that a Y chromosome pairs with both X's may be judged by the frequency that it directs both X's to the same pole. (Invariable formation of an XXY trivalent when XY pairing occurs, in contradistinction to XY bivalent and X univalent formation part of the time, offers the maximum frequency of X-nondisjunction for the minimal frequency of pairing and exchange interference by the Y. Although this interpretation may underestimate the effect of the Y, for the sake of simplicity it is adopted here.) If meiotic pairing occurs exclusively before exchange, the amount of secondary nondisjunction should be a gauge of the degree to which the Y interferes with crossing over between the X's throughout their length. As noted above, the possibility that the Y, acting only before exchange, effects a localized proximal reduction, is refuted by the fact that secondaries are nonrecombinants.

Table IV presents a comparison of exchange frequency in the X chromosomes in the presence (A) and absence (B) of a Y. When both X's are isosequential from yellow to the spindle fiber attachment (Cross I^1 A and B), the total amount of crossingover is significantly increased by the Y from 67.2 to 70.1 per cent. A regional examination of exchange (Table IV, column 3) discloses the increase occurs in the distal portion (y to v) and that in the proximal portion (v - y^+) crossingover is decreased. Since the distal increase exceeds the proximal

decrease, an increase in the total amount of exchange is observed. A classification according to crossover rank gives the following distribution:

	Total	Exceptions	0	1	2	3
XX	2433	0	1040	1154	234	5
XXY	2090	19	828	988	231	5

A tetrad analysis of the above data gives 4.73 per cent noncrossover tetrads among the progeny from XX mothers and 4.98 per cent from XXY mothers and indicates that the Y does not increase the frequency of noncrossover tetrads.

When the X chromosomes are heterozygous for In (I) B^{MI}, 22.3 per cent secondary nondisjunction occurs (Table IV, Cross II^1, column 2) and this frequency represents the expected decrease in X exchange when a Y is present. Examination of the experimental results (Table IV, Cross II^1, column 3) shows that crossingover is significantly increased by a Y from 27.93 to 32.54 per cent. The distal regions are again responsible for the increase.

In the case of the In (1) sc^7 heterozygote (Table IV, Cross III', column 2) 23.6 per cent secondary nondisjunction takes place. Crossingover is significantly decreased by the presence of a Y (Table IV, Cross III, column 3) from 34.65 to 31.82 per cent but not to the extent anticipated by the frequency of secondary nondisjunction (2.8 per cent actual vs. 8.2 per cent expected).

Reference to the pattern of crossingover in the isosequential X chromosomes serves to elucidate the results obtained with the inversion

heterozygotes. When no inversion is present, the Y increases exchange distally and decreases it, to a lesser extent, proximally. Introduction of the proximal inversion, B^{MI}, into one X, eliminates the region of reduction but retains the region of increase so that a greater net increase is observed than with the normal X's. In (I) sc^{7}, a distal inversion, eliminates the region of increase but retains the region of decrease, reversing the relationship so as to produce a net decrease. In no case is the decrease observed that predicted by a model that limits pairing to a single preexchange event.

Nonhomologous Association

If meiotic pairing occurs exclusively before exchange, the frequency of nonhomologous association should be a measure of the extent to which a nonhomolog competitively interferes with association between homologs. The best indication of the amount of homologous pairing is exchange frequency. It follows that the frequency of nonhomologous association should be negatively correlated with the frequency of exchange. The expression, $a = 1 - 2n$, where a = association and n = nondisjunction of the elements concerned, is used to calculate the frequency of nonhomologous association.[16] The value of "a" (Table I, column 4) is too low because one-half of the Y-2 segregations are nondisjunctional for the second chromosomes and lead to lethality. A "corrected" value for "a" is given in Table I, column 5.

The "corrected" value for "a" may also be incorrect for the following reason. The manner of segregation of the elements in the trans-

location quadrivalent (chromosome two crossover tetrads) and in the translocation trivalent (chromosome two noncrossover tetrads) will lead to an undetermined frequency of aneuploidy which could be different in the two situations. If less aneuploidy results from disjunction from a chain of four, exchange tetrads will be preferentially recovered and the value of "a" is underestimated. If less aneuploidy results from a chain of three, nonexchange tetrads will be preferentially recovered and the value of "a" is overestimated. Aneuploidy, in any case, is not expected to exceed 50 per cent. If we assume the chain of four produces 50 per cent aneuploid gametes whereas the chain of three produces no aneuploid gametes, we obtain a minimal value for "a" which turns out to be the original uncorrected value of about 50 per cent. Thus, it may be assumed the y^+ Y and the Gla chromosome associate at least 50 per cent of the time.

Crossover frequencies are given in Table II, column 4. For each cross, A represents the presence of y^+ Y and B its absence. A comparison of A and B shows that observed crossing over between homologs is not reduced in the presence of a Y. In one case (Cross I) a small, but significant, increase is noted; in the two remaining cases the values are very close. The observed recombination values may not reflect accurately the true recombination values. Translocation heterozygates invariably give rise to a certain frequency of aneuploid gametes. If the probability of occurrence of a euploid gamete is greater among the exchange tetrads than among the nonexchange tetrads, recombination will appear higher than it actually is; in the reverse situation it will

appear lower. Alteration of true exchange frequency, arising from this source, will only affect the present study of the proportion if euploidy among the recombinant class and the nonrecombinant class, for the second chromosome, is altered by the presence of the Y chromosome. In the case of the recombinant class, the probability of euploidy is expected to be the same whether the Y is present or absent, for the Y, as will be shown below, assorts independently of the translocation group and does not affect its mode of disjunction. In the case of nonrecombinants, the y^+ Y and the Gla chromosome segregate from one another independently of the translocation chain of three so as to produce gametes aneuploid for chromosome two one-half the time. If the Y is absent, the nonrecombinant Gla chromosome is expected to also assort independently of the translocation chain of three and cause aneuploidy 50 per cent of the time. The presence of the Y chromosome might be expected to cause aneuploidy among the nonrecombinants in excess of 50 per cent only if one element of the translocation fails to cross over with the normal three. Failure of crossingover is, however, expected to be low since the multiple inversion in the second chromosome will, as a consequence of the interchromosomal effect, increase crossingover in the uninverted arms. Thus, it appears that if the Y alters the proportion of euploidy among recombinants and nonrecombinant classes, the alteration could only be a slight one.

It should also be noted that if the actual exchange value is markedly decreased by the presence of the Y, in order for the observed values to be very close, as they are, the correction factors for recovery

of recombinants in the two situations must be reciprocals. Thus, if exchange in the presence of a Y is reduced 50 per cent, the probability of recovery of recombinants must be double that of the no Y situation. Since it has been pointed out that euploidy among recombinants should not be affected by the presence of the Y (see above), a twofold increase in recovery of recombinant progeny from XXY mothers as compared to XX mothers could only be accomplished by a very high frequency of lethality among the nonrecombinant class. That this is not the case is borne out by a comparison of fertility in the XXY and XX females. In order that the observed recombination value be twice the actual value, approximately 60 per cent of the nonrecombinant second chromosome gametes from XXY mothers must be aneuploid and lead to zygote lethality. Since it is known that the presence of a Y chromosome per se in females reduces fertility about 33 per cent,[18] the combined effects should reduce the number of progeny from XXY mothers to about one-third of normal. As single females were used in the present experiments, a comparison of the average number of offspring from the two genotypes can be made. The average number of progeny is 149 for XXY mothers and 186 for XX mothers indicating that the Y induces no increase in zygote lethality. This observation is confirmed by that of Oksala[8] for a parallel situation.

Although Cross II was marked so as to detect double crossovers, only singles were observed among some 5000 flies. It is apparent from Table II that the viability of the translocation-bearing progeny is depressed, particularly among the noncrossovers. As a consequence, reciprocal classes are unequal. The depression is more noticeable with

T (2;3) A and is greatest in Cross II, Exp. 1 when T (2;3) A carries all of the recessive mutants. The viability of the Gla and T (2;3) A homologues was brought closer to equality by transferring the mutants px and sp to the Gla chromosome (Cross II, Exp. 2). In the crossover classes, the discrepancy in viabilities is generally reduced when the mutants, through crossingover, are shifted to the inversion chromosome (Cross I and Cross II, Exp. 1).

A clarification of the role that a nonhomolog plays in the synaptic and exchange processes between homologs is obtained by examining the distribution of the nonhomologue (y^+ Y) with respect to the crossover and noncrossover progeny. These data (Table III) show that, in each situation the nonhomolog (1) is recovered randomly among the recombinants, (2) is recovered highly nonrandomly among the nonrecombinants. This means that only noncrossover tetrads participate in nonhomologous associations.

IV. DISCUSSION

If the chiasmata that are observed by cytologists during meiotic prophase are the equivalents of genetic crossovers, and if a chiasma is required for regular disjunction of homologs, crossingover should then be necessary for regular disjunction. Yet, a number of investigators have established that, at least in Drosophila females, exchange is not a prerequisite for disjunction. Evidence for this comes from normal and mutant individuals as well as those carrying rearrangements. Approximately 6 per cent of the X-tetrads of females with isosequential X chromosomes are calculated to be noncrossovers.[19] A similar experiment, described above (Table IV, Cross I), that included measurement of crossingover in the proximal heterochromatic regions, is in good agreement, giving a value of 4.7 per cent noncrossover X-tetrads. The frequency of X-nondisjunction in this situation is about one-hundredth of the frequency of noncrossover X-tetrads or approximately 0.05 per cent.[4] Cooper[5] has shown that noncrossover X-tetrads from inversion heterozygates may reach 80 per cent without appreciably affecting the segregation behavior of the X's. The fourth chromosomes in diploid Drosophila females undergo disjunction regularly in the absence of exchange and nonhomologues may disjoin with very high frequencies but never crossover. The mutant c III G practically eliminates crossingover but chromosome assortment shows a marked departure from randomness.[20]

These observations indicate that something other than exchange governs or modifies disjunctive behavior. Synapsis appeared to be the most likely possibility and this led Dobzhansky to postulate that intimacy of preexchange pairing determines disjunction. If such is the case, it is not readily apparent why a Y chromosome, possessing infinitely less homology for both X's of an inversion heterozygote than the X's possess for one another, should provide sufficient pairing intimacy to disjoin from them as frequently as 63 per cent. Even less rationale can be found for pairing and disjunction between nonhomologs.

The experiments described above proposed to determine first whether only noncrossover tetrads are involved in nonhomologous associations. The results clearly demonstrate that this is the case. In this respect such tetrads resemble secondary exceptions. Establishment of this point leads to two possible alternatives. If the disjunctive associations between nonhomologs or heteromorphs occur simultaneously with pairing and crossingover between homologs, these associations should produce noncrossover tetrads. In this event, the total amount of crossingover should be reduced. If, on the other hand, crossing over precedes the disjunction association between nonhomologs or heteromorphs, the total amount of crossingover is not expected to be reduced except to the extent the Y interferes with proximal X exchange. The experimental results indicate crossingover precedes disjunctive association. In no case does association between the $\underline{y}^+$ Y and chromosome two cause a decrease in exchange between the second chromosomes. In only one case does association between the Canton-S Y and the X chromosomes cause a

decrease in exchange between the X's. The decrease in this instance is considerably less than that predicted by the frequency of secondary nondisjunction and is attributable to the fact that exchange is adequately measured only proximally.

The difficulty in elucidating the interrelations among the meiotic events appears, thus, to have arisen from the omission of one of the steps in the process. Interpolation of a second pairing event, after exchange, provides a means of resolving such apparent inconsistencies as the facts that pairing is highly specific, yet nonhomologs may pair very regularly; that decrease in exchange leads to an increase in nondisjunction, yet exchange is not necessary for regular disjunction; that a nonhomolog or a heteromorph associates exclusively with noncrossover tetrads without apparently increasing the number of such tetrads.

The following sequence of meiotic events is postulated, (1) exchange pairing, (2) exchange, (3) distributive pairing, (4) disjunction.

Exchange pairing is a prerequisite for exchange but does not necessarily lead to exchange. It occurs between specific homologous loci. If more than two such loci are present it is competitive since at any particular level pairing is by two's. As has been demonstrated for X-chromosome duplications, a competitor may decrease crossingover considerably, yet rarely participate in a crossover.[9]

Distributive pairing is concerned with the segregation process. It occurs after crossingover is complete. Crossover tetrads remain associated; chromosomes or chromosomal elements not involved in a

crossover pair with one another. When more than two noncrossover elements are present, pairing is competitive.[16] Pairing of this kind, although perhaps affected by homology, involves nonhomologous elements as well.

Distributive pairing is considered to be operative both when the genome is normal and when rearrangements or aneuploids are present. Under normal conditions about 95 per cent of the X-tetrads and probably a larger percentage of the major autosomal tetrads are crossovers. For these, the pattern of distributive pairing is set by exchange. The residue, which are noncrossovers (about 5 per cent of the X-tetrads and an undetermined number of autosomal tetrads), and which would otherwise assort randomly, engage in distributive pairing. How important a role, if any, homology plays in this process is unknown. The coincidence of X and major autosome noncrossover tetrads in an oocyte should be the product of their occurrence singly and the infrequency of this event provides little opportunity for nonhomologous association.

When a Y chromosome is added to the normal genome, it competes for exchange pairing because of homology with the X's. Since the Y never participates in exchange, its effect is measurable only as a proximal decrease in crossingover between the X's. (A distal increase observed in the X's in the presence of a Y is unexplained.) The Y is again active at distributive pairing. At this time, it competes for noncrossover X-tetrads that would in its absence pair distributively with and disjoin regularly from one another and diverts a large portion of these into secondary exceptionals.

Different X-inversions, introduced heterozygously, will, depending on their size and location, possess varying degrees of effectiveness for reducing crossingover between the X's. The greater the number of noncrossover tetrads so produced and thus available for distributive pairing with the Y, the larger will be the fraction of the total tetrads that the Y diverts into secondary exceptionals.

Previously, association between nonhomologs at meiosis was attributed to the absence of adequate homologous pairing partners for them.[16] This concept may be more precisely defined now. Associations between nonhomologs at meiosis occur only after exchange and specifically between elements not participating in an exchange. The fourth chromosomes are an exception to this generalization, for although noncrossovers, they appear to have developed a secondary mechanism to assure regular disjunction. Experiments (unpublished) have shown that the fours continue to segregate regularly when an extra Y chromosome is added to the complement. They may be induced to associate nonhomologously when present as an extra element or when prevented by rearrangement from pairing with their homolog.[7]

Cooper[5] has speculated that structures such as telomeres and collochores, may have evolved for insuring regular disjunction in the absence of chiasmata. These accessory devices may function at distributive pairing.

These experiments provide no information concerning the exact time during which the meiotic events occur. The only evidence as to the time of crossing over in Drosophila comes from the work of Plough[21] and places

it at "the very earliest oocyte." Plough believes this probably corresponds to the fine thread stage (leptotene). If exchange is completed during leptotene, distributive pairing would be expected to occur sometime subsequent to this. Thus, Pontecorvo's[22] speculation that cytologically visible pairing is only a mechanical device necessary for segregation, may well turn out to be the case.

TABLE I

FREQUENCIES OF NONHOMOLOGOUS ASSOCIATION

Cross IA - Progeny from $y^2/y^2/y^+$ Y; Ins (2LR) Gla, Gla/T (2;3) 101, al sp^2 ♀ x y^2; al px sp/al px sp ♂

Cross IIA - Progeny from (1) $y^2/y^2/y^+$ Y; Ins (2LR) Gla, Gla/T (2;3) A, al Bl px sp ♀ x y^2; al px sp/al px sp ♂

(2) $y^2/y^2/y^+$ Y; Ins (2LR) Gla, Gla px sp/T (2;3) A, al Bl ♀ x y^2; al px sp/al px sp ♂

	y^+ Y	No y^+ Y	n (%)	a (%) Uncorrected	a (%) "Corrected"*
IA					
Ins (2LR) Gla	297	1168	$\frac{629}{2692}$ = 23.4	53.2	69.4
T (2;3) 101	895	332			
IIA Exp. 1					
Ins (2LR) Gla	206	787	$\frac{380}{1602}$ = 23.7	52.6	68.9
T (2;3) A	435	174			
IIA Exp. 2					
Ins (2LR) Gla	154	562	$\frac{305}{1208}$ = 25.2	49.6	66.3
T (2;3) A	341	151			

n = nondisjunction of y^+ Y and Ins (2LR) Gla.

a = association between y^+ Y and Ins (2LR) Gla.

*a "corrected" for aneuploids for chromosome two arising from ½ Y-Gla segregations.

TABLE II

EFFECT OF A NONHOMOLOG ON CROSSINGOVER BETWEEN HOMOLOGS

Cross I A, B - Progeny from y^2/y^2; Ins (2LR) Gla, Gla/T (2;3) 101, al sp^2 ♀♀ with (A) and without (B) a y^+ Y x y^2; al px sp/al px sp ♂♂

	Noncrossovers		Crossovers			
	T (2;3) 101	Ins (2LR) Gla	T (2;3) 101	Ins (2LR) Gla	Totals	% Crossingover
A (y^+ Y)	994	1226	233	237	2690	17.47 ± 00.73
B (No y^+ Y)	1834	2227	339	398	4848	15.20 ± 00.52

Cross II A, B - Progeny from (1) y^2/y^2; Ins (2LR) Gla, Gla/T (2;3) A, al Bl px sp ♀♀ with (A) and without (B) a y^+ Y x y^2; al px sp/al px sp ♂♂

(2) y^2/y^2; Ins (2LR) Gla, Gla px sp/T (2;3) A, al Bl ♀♀ with (A) and without (B) a y^+ Y x y^2, al px sp/al px sp ♂♂

	Noncrossovers		Crossovers			
	T (2;3) A	Ins (2LR) Gla	T (2;3) A	Ins (2LR) Gla	Totals	% Crossingover
Exp. 1						
A (y^+ Y)	496	887	113	106	1602	13.67 ± 00.86
B (No y^+ Y)	243	497	49	69	858	13.76 ± 01.18
Exp. 2						
A (y^+ Y)	412	591	80	125	1208	16.97 ± 01.08
B (No y^+ Y)	533	749	105	134	1521	15.71 ± 00.93

TABLE III

ASSORTMENT OF THE NONHOMOLOG (y^+ Y) IN THE CROSSOVER AND NONCROSSOVER PROGENY

Cross IA - Progeny from $y^2/y^2/y^+$ Y; Ins (2LR) Gla, Gla/T (2;3) 101, al sp^2 ♀♀ x y^2; al px sp/al px sp ♂♂

Noncrossovers				Crossovers			
T (2;3) 101		Ins (2LR) Gla		T (2;3) 101		Ins (2LR) Gla	
y^+ Y	No y^+ Y	y^+ Y	No y^+ Y	y^+ Y	No y^+ Y	y^+ Y	No y^+ Y
791	203	184	1042	104	129	113	124

Cross IIA - (1) Progeny from $y^2/y^2/y^+$ Y; Ins (2LR) Gla, Gla/T (2;3) A, al Bl px sp ♀♀ x y^2; al px sp/al px sp ♂♂

(2) Progeny from $y^2/y^2/y^+$ Y; Ins (2LR) Gla, Gla px sp/T (2;3) A, al Bl ♀♀ x y^2; al px sp/al px sp ♂♂

	Noncrossovers				Crossovers			
	T (2;3) A		Ins (2LR) Gla		T (2;3) A		Ins (2LR) Gla	
	y^+ Y	No y^+ Y	y^+ Y	No y^+ Y	y^+ Y	No y^+ Y	y^+ Y	No y^+ Y
Exp. 1	387	109	153	734	48	65	53	53
Exp. 2	317	95	91	500	24	56	63	62

TABLE IV

EFFECT OF A Y CHROMOSOME ON CROSSINGOVER AND DISJUNCTION OF THE X'S

Cross I' - Male progeny from $\underline{y^2\ sc\ v\ f} \cdot \underline{sc^{VI}\ y^+}/\underline{y^2\ cv\ wy\ car}$ ♀♀ with (A) and without (B) a Y^{C-S} x $\underline{y\ Hw\ B^{SV6}}$ ♂♂

Cross II' - Male progeny from In (1) $\underline{B^{MI}}/\underline{y^2\ v\ f}$ ♀♀ with (A) and without (B) a Y^{C-S} x XY, $\underline{yB}/Y^{C-S}$ ♂♂.

Cross III' - Male progeny from In (1) sc^7, $\underline{sc^7}/(\underline{y^2})^*\ \underline{v}\ (\underline{wy})^*\ \underline{f\ car}$ ♀♀ with (A) and without (B) a Y^{C-S} x XY, $\underline{yB}/Y^{C-S}$ ♂♂

	Total Males	% Nondisjunction	% Crossingover				
			Distal		Proximal		Total
I' A (Y^{C-S})	2090	1.82	($\underline{y^2}$-$\underline{wy}$)	46.45	($\underline{wy}$-$\underline{y^+}$)	23.64	70.09 ± 01.00
I' B (No Y^{C-S})	2433	.08	($\underline{y^2}$-$\underline{wy}$)	40.28	($\underline{wy}$-$\underline{y^+}$)	26.96	67.24 ± 00.95
II' A (Y^{C-S})	3038	22.3	($\underline{y^2}$-$\underline{v}$)	26.86	($\underline{v}$-$\underline{f}$)	5.68	32.54 ± 00.85
II' B (No Y^{C-S})	4688	.06	($\underline{y^2}$-$\underline{v}$)	22.38	($\underline{v}$-$\underline{f}$)	5.55	27.93 ± 00.66
III' A (Y^{C-S})	4994	23.6	($\underline{y^2}$ or $\underline{sc}$-$\underline{v}$)	6.63	($\underline{v}$-$\underline{car}$)	25.19	31.82 ± 00.66
III' B (No Y^{C-S})	4707	.04	($\underline{y^2}$ or $\underline{sc}$-$\underline{v}$)	5.65	($\underline{v}$-$\underline{car}$)	29.0	34.65 ± 00.69

*( ) designates present in some crosses.

V. SUMMARY

1. To determine if crossover as well as noncrossover tetrads participate in nonhomologous associations, nonrandom assortment between a $\underline{y}^+$ Y and a multiple inverted second chromosome, Ins (2LR) Gla, has been studied at the same time that crossingover between Ins (2LR) and its translocated homolog T (2;3) A or T (2;3) 101, has been followed. The results show that only noncrossover tetrads participate in nonhomologous associations.

2. Crossingover between Ins (2LR) Gla and its translocated homolog, T (2;3) A or T (2;3) 101, has been measured when the inverted chromosome is participating in nonhomologous association with a $\underline{y}^+$ Y and when the $\underline{y}^+$ Y is absent. The frequency of crossingover appears not to be decreased by the occurrence of nonhomologous association. It is concluded that nonhomologous associations do not produce noncrossover tetrads but that these associations take place between noncrossover tetrads after exchange.

3. Crossingover between $\underline{y}^2$ $\underline{sc}$ $\underline{v}$ $\underline{f}$.$\underline{sc}^{VI}$ $\underline{y}^+$ and $\underline{y}^2$ $\underline{cv}$ $\underline{wy}$ $\underline{car}$, between In (1) B^{MI}, $\underline{cm}$ $\underline{B}^{MI}$ and $\underline{y}^2$ $\underline{v}$ $\underline{f}$ and between In (1) sc^7, $\underline{sc}^7/(\underline{y}^2)$ $\underline{v}$ ($\underline{wy}$) $\underline{f}$ $\underline{car}$ has been measured in sisters that carry or fail to carry an extra Canton-S Y chromosome. In no case does the Y chromosome decrease crossing over to the extent it causes X nondisjunction. A proximal decrease is observed, but in two of the three cases studied total crossingover is increased in the presence of Y. It is concluded that the Y does not appreciably affect the number of noncrossover X-tetrads

but that associations take place between noncrossover X-tetrads and a Y chromosome after exchange leading to secondary nondisjunction.

4. It is postulated that the sequence of meiotic events is (1) exchange pairing, (2) exchange, (3) distributive pairing, (4) disjunction. Exchange pairing occurs between specific homologous loci; it is competitive if more than two such loci are present; it is a prerequisite for exchange but it does not necessarily lead to exchange. Distributive pairing occurs after exchange; crossover tetrads remain associated; noncrossover elements pair with one another; if more than two noncrossover elements are present, pairing is competitive; it may be influenced by homology but it involves nonhomologous elements as well.

5. The application of this model to the situation in normal females, to secondary nondisjunction and to nonhomologous association is discussed.

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