NORMAL SEGREGATION WITHOUT CHIASMATA IN FEMALE DROSOPHILA MELANOGASTER

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FOR most chromosomes, segregation at meiosis depends upon an association or conjunction of homologs at metaphase of the first meiotic division. Generally the required state of metaphase conjunction is itself dependent upon an antecedent association or synapsis of homologous chromosomes during the inception of meiotic prophase. Following the onset of synapsis, homologous chromosomes may undergo reciprocal breakage and a new union of non-sister chromatids with consequent genetic crossing over and the formation of chiasmata. The hypothesis that the chiasmata observed by the cytologist are the direct consequences of such interchanges or crossing over was first put forth by JANSSENS (1909, 1919a, 1919b, 1924). WILSON and MORGAN'S (1920) critical review of JANSSENS' hypothesis in effect drafted our present "chiasma-type theory," while DARLINGTON (1930, 1931a) supplied the first rigorous cytological proofs that crossing over gives rise to chiasmata and that every chiasma represents an exchange of partners between non-sister chromatids within bivalents and multivalents.

Following the events of crossing over, the chromosomes attain their condensed state by the completion of their meiotic coiling and the acquisition of accessory substances (or "matrix") and perhaps also a pellicle. While at metaphase the bivalent has its definitive form, yet the mechanism by which the homologous chromosomes are held together and thus have their coorientation on the spindle assured remains difficult to elucidate. It is conceivable that homologous chromosomes which have synapsed may remain associated until metaphase by a persistence of the "forces" which brought about the earlier synapsis, or by chiasmata, or by the development of partially-common matrices or pellicles, or by some mechanism associated with the coiling process, or by some new agent developed during prophase, or perhaps by a combination of two or more of these possible binding agents. Nevertheless, as is well known, cytologists have focused their attention almost exclusively on the possible role of the chiasma in guaranteeing conjunction and therefore segregation at meiotic metaphase, other factors having been for the most part overlooked or ignored.

The development by DARLINGTON (1931a, 1932) of a simple mechanical interpretation of the basic differences between meiosis and mitosis (the so-called "precocity hypothesis") led to the most vigorous advance and stimulating period of research that cytology has known since 1900. But one characteristic of that period of cytogenetic synthesis was a narrow preoccupation with only those structures, events, and assumptions which are directly involved in

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the hypotheses formulated by DARLINGTON and his disciples. By the rule of logical parsimony, of all the possible mechanisms of conjunction at meiotic metaphase only the chiasma is required by the propositions of the precocity hypothesis. Thus the "chiasma hypothesis of metaphase pairing" became a corollary of the precocity interpretation of meiosis. This corollary, it should be emphasized, seemed well substantiated for the case of large chromosomes by observations upon multivalent formation in polyploid liliaceous plants (DARLINGTON 1929, 1931a, 1931b and later). Where chromosomes proved too small or too poorly fixed for critical study, on the other hand, little hesitance or reserve has been shown by most authors in deducing *ex hypothesi* that chiasmata are providing the mechanism of metaphase conjunction. Although the precocity hypothesis is now known to be in manifest disagreement with the observations of a large number of independent workers, and is accordingly no longer a valid tool for further research (SAX 1936; HUSKINS 1937; COOPER 1938; BAUER 1939; HUGHES-SCHRADER 1940; SCHRADER 1944; and others), its ancillary concept which holds the chiasma to be the essential condition for segregation remains one of the most widely applied notions in cytogenetics. In spite of the fact that there is now a fairly impressive list of forms in which chiasmata are not necessary for segregation in at least one sex (see HUGHES-SCHRADER 1943a, 1943b; COOPER 1944), the general assumption of the DARLINGTON school now appears to be that chiasmata are universally essential for segregation in the meiosis of at least the opposite sex of these exceptional forms. Thus one sex is assumed to have developed a new or special mechanism of metaphase conjunction, whereas the other sex is held to retain the assumedly primitive method of conjunction by chiasmata. Because such a line of reasoning inexorably demands that crossing over underlies meiosis and sexual reproduction, it is not surprising to find that DARLINGTON (1939, p. 16) holds the view that "It is the central fact of genetics."

But are crossing over and the resultant chiasmata invariably necessary for orderly segregation in at least one sex of these forms? If not, the chiasma hypothesis of metaphase pairing certainly becomes suspect for all forms with small chromosomes, the precise details of which are difficult to ascertain, or whose chromosomes have been only casually investigated. The most promising organism for an investigation of this question seems to be *Drosophila melanogasier*. It is fairly definitely known that in this and in related species (*D. pseudoobscura*) crossing over and chiasmata either do not occur at all or are exceedingly rare in meiosis of the male (review and analysis in COOPER 1944). In the female a satisfactory cytological study of meiosis appears unfeasible at the moment, but the problem may be very elegantly attacked by purely genetic methods in this sex. On the basis of the chiasma hypothesis of metaphase pairing, approximately fifty percent of all non-crossover tetrads should give rise to non-disjunctional gametes with the consequent appearance of a proportional number of matroclinous female and patroclinous male exceptions among the offspring. GERSHENSON (1935), STONE and THOMAS (1935), and STURTEVANT and BEADLE (1936), studying segregation in X-chromosome inversion-heterozygotes, maintain that although in certain of their crosses there occurs

a very considerable percentage of noncrossover tetrads (hence chiasma-free bivalents), there is no corresponding increase in the matroclinous female exceptions above the normal rate. The observations of STURTEVANT and BEADLE alone would have disposed of the chiasma hypothesis as a reliable basis for general deduction were it not for the fact that the small right arm of the X (KAUFMANN 1934; PROKOFIEWA 1935) was not genetically marked in their experiments. It therefore remained possible that undetected exchanges or chiasmata in the right arm were actually guaranteeing normality of segregation in these females. The experiments described below show that this is not the case and that normal segregation of the sex chromosomes of female *Drosophila melanogaster* is independent of crossing over, as STURTEVANT and BEADLE, GERSHENSON, and STONE and THOMAS concluded.

METHODS

The experiments were all conducted at 25° ± 0.2°C. Single virgin females heterozygous for the two special X chromosomes described below were mated in shell vials with one (or rarely, two) males for a 24-hour period. The couples were then transferred without etherization to freshly yeasted half-pint bottles containing a non-agar, cream of wheat and molasses food prepared according to the directions of SPASSKY (1943). For the most part, the flies did very well on this medium, although occasional bottles turned soupy and yielded relatively small progenies. As soon as pupae appeared, the parents were removed from the bottle. Flies were counted daily from onset of eclosion of the adults until the end of the period of emergence.

Egg collections from single females and rearing of flies from the eggs were carried out in the following manner. Glass rings 18 mm in external diameter by 4 mm in depth were cut from soft glass tubing. Ordinary microscope slides were cut into rectangles 20 mm × 45 mm in size. An area sufficiently large to encompass the base of one of the glass rings was then painted with a melted mixture of vaseline and soft paraffin near the middle of the slide. A glass ring was pushed gently down on the warm wax so that a water-tight cylindrical cell about 4 mm deep was formed. Ordinary food blackened with washed animal charcoal was run into the cell in sufficient quantity to give a smoothly rounded mound of food rising above the rim of the glass ring. When hard, the surface of the food was painted with a thin yeast suspension, care being taken to keep the yeast from wetting the rim of the cell. The glass slip carrying the food was then placed in an 80 mm × 20 mm shell vial, and a previously mated female and male were transferred into it without etherization. Thereafter the vial was plugged with cotton, and during the egg laying period placed on its side, food uppermost, in the incubator.

Flies were transferred without etherization to vials containing fresh cells of food at the end of each 12- or 24-hour interval of egg laying for a period of six days, at the end of which time the egg collections were arbitrarily terminated. Eggs were counted by means of a dissecting microscope immediately following transfer of the parents by removing the slide from the vial. After the first

counting of the eggs, the slide was returned to the vial. A drop of distilled water was then run into the vial in such a way that it was held by capillarity along the lower borders of contact of the glass slip with the wall and bottom of the vial. By this means excellent humidity conditions were preserved during hatching of the eggs in spite of the fact that only a cotton plug was used. About 25 to 30 hours after the initial egg count, the numbers of inviable eggs were determined. Thereafter the glass ring with the contained food and larvae was slipped off the slide and placed in a freshly yeasted half-pint bottle of food. In this way there was virtually no handling of eggs or early larvae, a fact which may help account for the surprisingly high viability shown by the figures of table 2.

DESIGN AND RESULTS OF THE EXPERIMENT

Two special X chromosomes were made up in stock for these experiments (fig. 1). One of them contains both the delta-49 (*dl-49*) and B^{M1} inversions,

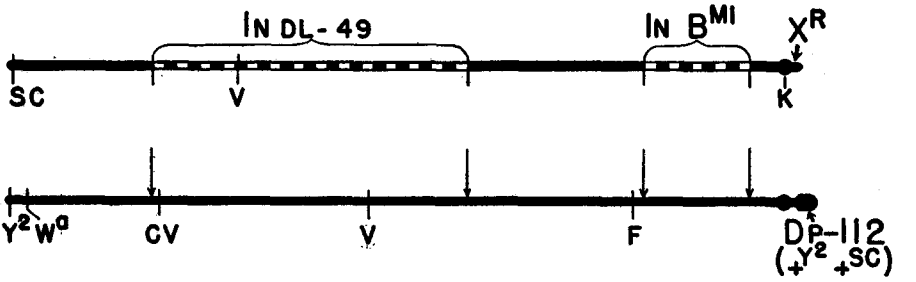

FIGURE 1. Maps of the X chromosomes used. The loci of the genes involved and the approximate limits of the inversions are as follows: y^2(0.0), *sc*(0.0+), *cv*(13.7), *v*(33.0), *f*(56.7), *Indl-49*(12± −41±), InB^{M1}(57± −67±). The actual sizes of X^R and *Dp112* are not known, but it may be taken for granted that they are very small. DOBZHANSKY (1932) states the cytological length of *Dp*(1;*f*)*112* to be equal to or smaller than the diameter of the fourth chromosome. The kinetochore is labelled k, and the vertical arrows pointing to sections of the *Dp112* chromosome indicate the approximate endpoints of the inversions in the other X.

while the other has a small duplication attached to the right arm (X^R) of the X. Both chromosomes carry the recessive mutant vermilion (*v*), so that when females carrying both these chromosomes are crossed to non-vermilion males all matroclinous female exceptions in the offspring are readily detected by their vermilion eyes. Crossing over between these two X's can be determined by the phenotypes of the regular male offspring Since the marking genes to the left of *dl-49* are different in the two chromosomes, crossovers in this region are readily detected, except for simple exchanges of scute (*sc*) for yellow^2 (y^2) into the chromosome bearing duplication 112 (*v. infra*). Crossveinless (*cv*) and forked (*f*) in the noninverted chromosome make it possible to detect exchanges between the two inversions. Inversion B^{M1} itself is associated with a weak Bar effect and so provides its own marker. Since y^2 at the left end of the chromosome bearing the duplication and *sc* at the left end of the inverted chromosome

are both covered by their wild type alleles in duplication 112 (*Dp112*), detection of exchanges to the right of inversion B^{M1} (that is, between B^{M1} and the kinetochore, or within X^R) is reliably accomplished.

As STURTEVANT and BEADLE (1936) have shown, single exchanges within inversions will not ordinarily be recovered, even though they may be expected to occur. Such single exchanges most probably result in a chromatid bridge and an akinetic fragment at the first meiotic division. At the second division the non-crossover chromatids of such bivalents may be expected to pass to the innermost and outermost nuclei in the egg. The egg nucleus, therefore, receives a single non-crossover strand. Double exchanges presumably do not occur within either inversion.

The importance of the heterozygous inversions in these experiments is owing to their general effect in cutting down crossing over along considerable lengths of the chromosomes, as was first postulated by STURTEVANT (1921). Within *dl-49* only 12 percent chiasmata (that is, 6 percent crossing over) are to be expected on the basis of STURTEVANT and BEADLE'S (1936) studies of attached-X females heterozygous for *dl-49*. No comparable estimate is available for inversion B^{M1}. But inasmuch as B^{M1} is only about ten map units long (see SUTTON 1943), it may confidently be expected that the frequency of crossing over will be at least halved and that at most 10 percent chiasmata will occur in this region. Accordingly not more than 22 percent (as a generous estimate) of all bivalents are expected to be conjoined by chiasmata occurring *within* the inverted regions.

Crossing over to the left of *dl-49* in females heterozygous for *sc v*, *dl-49*, B^{M1} can be estimated from a total of 15,390 flies from various experiments involving both XX and XXY females. Of 8,424 males obtained from *sc v*, *dl-49*, $B^{M1}/y^2\ w^a$ *cv v f*, *Dp112* females by *B*, Swedish-b or Oregon R males, only four were crossovers in this region. Of these only one male carried an exchange in the region between *sc* and w^a. Since 50 percent of such crossovers cannot be detected (that is, y^2 . . . *Dp112* and *sc* . . . *Dp112* are phenotypically indistinguishable), one may assume that perhaps another crossover or so in this region has been missed—although the addition of another exchange to this crossover class will hardly affect the calculated frequency of exchange to the left of *dl-49*. An additional 6,966 flies gave a total of eight males which were crossovers in the region between *sc* and *dl-49*, and in none of these cases were the reciprocal classes unclassifiable. At all events, no more than 13 crossovers to the left of *dl-49* need be accredited in a total of 15,390 flies in which such an exchange could be detected. This gives a crossing over value equal to approximately 0.08 percent. Such a value is far below that of STURTEVANT and BEADLE (1936) for heterozygous *dl-49*. They estimate about 0.5 percent crossing over in this region. Accepting their figure as a possible maximum, no more than 1 percent of all bivalents in the present experiments could have been conjoined by chiasmata to the left of *dl-49*.

Crossing over in the region between the two inversions is almost negligibly rare. Although the map length of this region is approximately 16 units, at most

three crossovers were obtained in a total of 15,390 flies in which such exchanges could be detected. This gives a crossing over frequency of about 0.001 percent. To the right of B^{M1} there were but two crossovers in the same total. In these experiments, therefore, considerably fewer than 1 per cent of the bivalents would be expected to have been conjoined by chiasmata occurring either between the inversions or to the right of B^{M1}. Totalling the exchange percentages for each region—both outside and inside of inversions—no more than 24 percent of all X-chromosome bivalents in *sc v, dl-49,* $B^{M1}/y^2\ w^a$ *cv v f, Dp112* females are expected to be held together at metaphase by chiasmata.

According to the chiasma hypothesis of metaphase pairing, the remaining 76 percent of the bivalents which are without chiasmata would fail to remain associated until metaphase or to coorient, and the resulting univalent X's

TABLE 1

Data from matings of sc v, Indl-49, In$B^{M1}/y^2\ w^a$ *cv v f, Dp112 by various males; an asterisk* (*) *indicates that the offspring recorded are derived from egg viability tests.*

MALE PARENT	TOTAL OFFSPRING	TOTAL ♀♀	EXCEP. ♀♀	TOTAL ♂♂	EXCEP. ♂♂	CROSSOVER ♂♂	FREQ. NONDISJUNCT.
ec dx	525	266	1	259	0	0	0.38%
*B**	532	265	0	267	0	0	—
B	2,749	1,424	0	1,325	1	0	0.08%
Swed-b*	1,628	824	3	804	0	0	0.18%
Swed-b	1,694	867	3	827	4	0	0.81%
Ore. R*	3,396	1,798	5	1,598	3	0	0.47%
Ore. R	6,236	3,414	2	2,822	4	4	0.19%
Totals	16,760	8,858	14	7,902	12	4	0.31%
Expected			2,078		1,854		38.0 %

should segregate at random. Thus 50 percent of the egg nuclei resulting from random segregation would be expected to contain one of the X's only, 25 percent would be expected to contain both X's, and the remaining 25 percent should contain neither X chromosome. Fertilizations of the aneuploid eggs will result either in inviable zygotes or in exceptional offspring, depending on the constitutions of the aneuploid egg nucleus and fertilizing sperm (BRIDGES 1916). An egg whose nucleus contains both X chromosomes will give a matroclinous exceptional female when the fertilizing sperm brings in a Y chromosome. Patroclinous male exceptions will occur as sterile XO individuals resulting from fertilizations of the no-X eggs by X-bearing spermatozoa. Most of the XX eggs fertilized by X-bearing spermatozoa will die in the pupal stage, only a few surviving through eclosion and emerging as superfemales. Finally the no-X eggs fertilized by the Y-bearing sperm will die. Since but 24 percent of the X-chromosome synapses of *sc v, dl-49,* $B^{M1}/y^2\ w^a$ *cv v f, Dp112* females are expected to give bivalents at first metaphase, there should be produced in

these experiments approximately 11.7 percent matroclinous female exceptions and an equal percentage of patroclinous male exceptions among the adult offspring. Approximately 23.46 percent of the flies of any one sex should be exceptions. The data of table 1 show no agreement with these predictions based upon the chiasma hypothesis of metaphase pairing. There is more than a hundredfold difference between the expectation and the observation. Segregation was essentially normal, there being no significant number of exceptional flies, in spite of the predominance of chiasma-free bivalents.

A supplementary check on these results was carried out to test whether or not there is a high zygotic inviability which could possibly account for the absence from these experiments of the large numbers of adult exceptions expected on the basis of the chiasma hypothesis of metaphase pairing. Table 2 gives the results of these tests of viability, and the crosses marked with asterisks in table 1 record the data on the adults from the same experiments. These findings show that the egg and larval-pupal mortality tests gave the same results as had been obtained from matings in which eggs had not been collected. They *prove* that there is no zygotic inviability which can account for the observed disagreement with the expectations based upon the chiasma hypothesis of metaphase pairing. Crossing over and chiasmata may accordingly be viewed as not necessary requirements for regular disjunction of the X chromosomes of *Drosophila melanogaster*.

TABLE 2

Egg and larval-pupal mortality for inversion heterozygotes; sc v, Indl-49, $InB^{M1}/y^2\ w^a$ cv v f, Dp112 by various males.

MALE PARENT	TOTAL EGGS	INVIABLE EGGS	% INVIABLE	HATCHED EGGS	ADULT FLIES	% EMERGENCE OF HATCHED EGGS
B	577	26	4.5	551	532	96.6
Swed-b	1,691	40	2.4	1,651	1,628	98.6
Ore. R	3,573	148	4.1	3,425	3,396	99.1
Totals	5,841	214	3.7	5,627	5,556	98.7
Expected		555	9.5	5,286	4,731	89.5

Comment on the egg-larval-pupal mortality experiments

The first egg collections to be made in these experiments were those of the series in which Oregon R males were used (table 2). Although the egg-inviability of 4.1 percent is by no means an extraordinarily low percentage (STURTEVANT and BEADLE (1936) record 3.1 percent inviable eggs for $dl\text{-}49/+\times B$, and 1.3 percent inviable eggs for $y^{2s}/B\times B$), the 99.1 percent recovery of adults from the hatched eggs awakened immediate suspicion. It seemed possible that some eggs had been overlooked in the initial counts, and thus the percentage emergence of adults recorded for the hatched eggs could be entirely too high. Accordingly, for this and other reasons the *B* and Swedish-b series were run, especial care being taken to avoid a miscount of the eggs. These ex-

periments show essentially the same results as the Oregon R series—namely, low egg mortality and high adult yields from hatched eggs (table 2). There may still be a small undetected error, but the confidence limits for P=0.99 indicate that for these experiments about 95 percent of all of the eggs from the inversion heterozygotes hatch, and that over 97 percent of the hatched eggs develop into adults.

DISCUSSION

The improbability of a chiasma interpretation of segregation in inversion heterozygotes

The following facts derivable from the experiments described above seem indisputable. (1) The great preponderance of X-chromosome tetrads formed by synapsis of *Indl-49*, InB^{M1} and *Dp112* chromosomes are not associated by simple exchanges or by chiasmata in either the long left limb (X^L) or in the minute right limb (X^R). (2) In spite of the high frequency of non-exchange tetrads ($\geqq 76$ percent), or bivalents not conjoined by chiasmata, segregation of the X chromosomes is essentially normal in the female. These facts are jointly opposed to the chiasma hypothesis of metaphase pairing. STURTEVANT and BEADLE (1936), GERSHENSON (1935), and STONE and THOMAS (1935) from similar experimental results have concluded that exchanges (hence chiasmata) are not necessary for normal disjunction of the X chromosomes in female *Drosophila melanogaster*. Probably but one serious criticism of the collective results from the study of heterozygous inversions in female Drosophila, and the conclusions drawn therefrom, is likely to be voiced in defense of the chiasma hypothesis of metaphase pairing. It may be contended by some that the designs of these experiments do not eliminate the possibility that undetected reciprocal exchanges in the so-called inert regions proximal to the kinetochores may in fact have been the causal agents of segregation in otherwise apparently non-crossover bivalents.[2]

[2] MATHER (1944) has recently published data which he contends require for their interpretation the regular occurrence of reciprocal chiasmata between X and Y in the male. For the following reasons MATHER neither establishes support for the reciprocal chiasmata hypothesis, nor demonstrates a "polygenic" constitution for the Y chromosomes studied.

(1) The genetic technique employed by MATHER is outmoded and gives uncertain isogenicity at the fifth generation with which he initiates his experiments. The expression $(1-2^{-n})^3$ for calculating the chance of isogenicity for all three autosomes after n generations of backcrossing is strictly applicable only if a single male is used per culture. MATHER's account makes clear only that at least two males per culture were used at the fifth and succeeding backcrosses. A *minimum* of ten males, therefore, were selected from the fourth generation for backcrossing of the experimental Y-chromosome stocks, producing the fifth or initially measured generation. If single males were employed before the fifth backcross, then the probability (P) that at least three males among the parents of the fifth generation carry one or more unreplaced autosomes is:

$$1-[p^{10}+10p^9(1-p)+45p^8(1-p)^2],$$

where $p=(1-2^{-4})^3=0.82$. Hence $P=0.25$, or the chance is 1 in 4 that at least two Y-chromosome strains may be expected to produce sons having one or more unreplaced autosomes at the fifth generation.

On the other hand, if in fact two males per culture were employed in every backcross, and if

Both GERSHENSON and STURTEVANT and BEADLE considered the remote possibility of explaining their results by such undetectable exchanges, and rejected such an interpretation as highly improbable. GERSHENSON's analysis consisted of showing that such reciprocal exchanges could be held accountable for normal segregation only by ignoring the data then available for crossing over to the right of bobbed, and that synapsis of *InClB*/+ chromosomes probably for the most part interferes with the pairing of the proximal regions. STURTEVANT and BEADLE, however, supplied three sets of data which collectively seem more than sufficient to exclude the possibility of reciprocal exchanges between the proximal inert regions. *InDf(bb)* carries an inverted segment extending from between *rb* and *rg* to between *car* and the kinetochore (STURTEVANT and BEADLE 1936). This chromosome furthermore possesses a deficiency for the proximal third of the somatic X chromosome, including the locus of bobbed (SIVERTZEV-DOBZHANSKY and DOBZHANSKY 1933). In spite of the loss of most of the region in which the supposed reciprocal exchanges are to occur, in spite of an upset of homology in the remaining fraction of the inert region by the included inversion, and in spite of the inevitable occurrence of non-exchange tetrads owing to the inversion, *InDf(bb)*/+mothers gave no matroclinous daughters in a total of 1,244 female offspring. In this instance it would be nothing short of fantasy to maintain that reciprocal exchanges in the deleted and partially inverted proximal region underlie normal segregation of the X chromosomes.

Less striking but additional supporting evidence that reciprocal exchanges are not involved follows from STURTEVANT and BEADLE's studies of the *sc-8* inversions. *Insc-8* and *InDf(sc-8)* likewise upset homology well within the inert region to the right of *bb*. Such an upset, from what is known concerning crossing over in regions immediately adjacent to inversions, must necessarily

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isogenic and heterogenic males breed alike, then the chance (p_n) that both male parents of a given strain are isogenic for all three autosomes at generation n is:

$$\left[1-\frac{5\times 4^{n-2}}{3\times 8^{n}}\right]^{3}$$

From this it follows that the chance that any one strain (such as, Y^{Ok}) is isogenic at the fifth backcross is 0.72, and the chance that two or more strains have one or more unreplaced autosomes is 2 in 5. In either case there is a very great likelihood that MATHER's Y-chromosome stocks were not isogenic at the start of his experiments.

(2) There is an evident lack of adequate environmental control.

(3) MATHER (page 320, line 4) states that there is no "external evidence" that unreplaced autosomes produce different effects in the two sexes. It should also be noted that there is likewise no external evidence for bristle modifiers in the Y chromosomes of his stocks or for reciprocal exchanges between X and Y.

(4) Internal contradictions, such as the failure of Y^{Or} to change $\widehat{XX}$ chromosomes in 22 generations (expt. 4) of association, exist within experiments.

(5) Theoretical considerations (even ignoring the weighty objections to constant reciprocal exchanges), such as the fact that both arms of Y pair with X,—and presumably with different frequencies (NEUHAUS 1936, 1937),—likewise shed grave doubts on the validity of MATHER's conclusions.

interfere with, and markedly lower, crossing over in the proximal segment. If reciprocal chiasmata are supposed to occur in this proximal segment, it must also be recognized that the *sc-8* inversions will have a suppressive action upon them. Accordingly, some percentage of the bivalents which would be expected to be conjoined solely by reciprocal chiasmata must in fact have no chiasmata occurring between the homologs. These, on the chiasma hypothesis, must fail to coorient and thus assort at random, with the result that 25 percent of their segregations terminate in the production of eggs which will give rise to matroclinous daughters when fertilized by Y-bearing spermatozoa. Experimentally it is found, however, that *Insc-8*/+ and *InDf(sc-8)*/+ give no higher frequency of exceptional daughters than does a normal female (0.02 percent for *Insc-8*/+; 0.31 percent for *InDf(sc-8)*/+; total, more than 5,000 female offspring). The same general disagreement with a chiasma interpretation of segregation was obtained in the present study in which InB^{M1} likewise upsets homology within the inert region (SUTTON 1943). In spite of this, no more than 0.16 percent exceptional daughters actually occurred among the 8,858 female offspring.

There seems to be but one conclusion to be drawn which is in harmony with the experimental results—namely, chiasmata are *not* a necessary condition for segregation in female Drosophila, although when present in a bivalent they may be a sufficient cause of segregation. Whatever the normal or average architecture of a bivalent may be in female Drosophila homozygous for normal or inverted chromosomes, it seems certain that the preponderance of bivalents in *Indl-49*, $InB^{M1}/Dp112$ and other inversion heterozygotes may be without chiasmata and nevertheless undergo normal disjunction.

The modes of conjunction at meiosis

Any mechanism which makes possible almost invariable separation of homologous kinetochores (hence chromosomes) to alternative cells at meiosis will guarantee segregation. There can be little doubt that crossing over with resulting chiasma formation supplies a means for doing just this. But neither can there be any doubt that the primary mechanism which brings about synapsis itself may likewise provide a device ensuring segregation if the paired condition is retained until metaphase. Very possibly the paired synaptic state can persist to metaphase in organisms having very small or little-coiled chromosomes at meiosis, whether or not chiasmata are formed. The principal significance of crossing over is that it provides both a mechanism giving recombination of genes as well as a means for preventing too great genetic divergence of initially identical chromosomes.

The assurance of segregation by mechanical ties through persistent chiasmata is almost certainly a secondary attribute. If, in evolution, increasing complexity in structure, or physiology, or even increasing bulk of a chromosome were to interfere with an established mode of conjunction derived from the synaptic mechanism, then there would be positive selection for any sec-

ondary mechanism or mechanisms which would serve to make the new karyotype stable in its meiotic behavior. Thus persistence of chiasmata, increase in shared matrix or pellicle, development of adhesive mechanisms (as telomeres and collochores may prove to be), or exploitation of what appears to be pairing by heteropycnotic structures—all such devices could serve in achieving persistent regularity of coorientation and segregation in spite of increasing divergence of the chromosome from its ancestral prototype and behavior. At first glance the chiasma appears to be the most likely mechanism to be fostered by such selection owing to the general ubiquity of crossing over at meiosis. Yet where is found the metaphase chromosome which is devoid of matrix and other components presumably accessory to the genonema and which by modification may serve as segregative mechanisms at meiosis? Since there are organisms in which crossing over is suppressed in one sex, it is clear that chiasmata are not alone in possessing the virtue of being capable of ensuring regularity of segregation. It is equally clear that selection has preserved devices guaranteeing segregation without the mediation of chiasmata, as has been here proven for the Drosophila female, even in organisms where crossing over is the rule. There seems little reason, therefore, to ignore the mechanisms of conjunction which assuredly replace or supplement chaismata in mechanically ensuring segregation in many organisms. To reason to a universal chiasma hypothesis has proven as unsound as the nineteenth century attempt to rigidly define a substance "protoplasm." It is no surprise that the chiasma proves only to be a sufficient—not a necessary—cause for segregation in some organisms, while in others, such as the lilies, of all possible mechanisms only chiasmata appear to be capable of guaranteeing segregation. What is surprising is the fact that alternative mechanisms which have been so carefully studied and described by the morphological school of cytologists have been so unwisely ignored or even discredited by many geneticists and cytogeneticists without further investigation.

SUMMARY

Approximately 76 percent of the bivalents formed by *Indl-49*, $InB^{M1}/Dp112$ X-chromosome heterozygotes are not conjoined by chiasmata.

In spite of the high frequency of non-exchange tetrads, or bivalents not conjoined by chiasmata, segregation of the X chromosomes is essentially normal in the female.

The chiasma hypothesis of metaphase pairing is not universally applicable, even if the domain generalized by it is specified in such a manner that aberrant forms of meiosis are deliberately excluded.

Mechanisms, other than chiasmata, which may serve to guarantee meiotic conjunction and segregation are briefly discussed.

Recent experimental work interpreted on the basis of the reciprocal chiasmata hypothesis of X-Y conjunction at meiosis in Drosophila males is shown neither to require nor to support that hypothesis.

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