

THE MECHANISM OF CROSSING-OVER

II

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IV. THE MANNER OF OCCURRENCE OF CROSSING-OVER

A. Interference

As soon as it seemed probable that the factors were linked in line, and that crossing-over was the actual method of interchange, it became of interest (so the writer believed) to analyze the exact mode of incidence of the interchange. The questions suggested themselves, for example, what was the total frequency of crossing-over, did any factors separate more often than they remained together, how often did crossing-over occur at two points simultaneously, and was there any tendency, in such cases, for the two points of crossing-over to be a definite distance apart, or in definite positions, etc. For answers to these questions might throw light on the mechanism of crossing-over, what cytological phenomena it was connected with, and what stage in synapsis it occurred at.

With these points in view the author calculated the linkage relations that would result on several possible schemes of interchange. The simplest possibility was that the chromosomes always twisted in loops of fixed length, though not of fixed position, and always underwent breakage, with recombination of homologous strands (*i. e.*, "crossed-over" in the technical sense), at each place that the strands crossed one another. In such a case there would always be a definite distance between one point of crossing-over and another; moreover, all factors which were separated by a distance great enough for double crossing-over to occur between them, *i. e.*, by the length of at least one loop, must always have either double or single (or multiple) crossing-over between them. Sturtevant's data, however, showed that this was

not true, and accordingly it had to be concluded that the length of the loop was variable, or that "crossing-over" did not always occur where the strands crossed.

Another possibility was that crossings-over were quite independent of one another, having an entirely random or chance distribution in the chromosome, with reference to each other. This would mean that when crossing-over occurred at one point, another crossing-over would be just as likely to occur coincidentally at any other given point—whether this be very near or far away—as when no crossing-over took place at the first point. But this latter scheme would not be that expected on the method of crossing-over proposed by Jannsens and followed by Morgan, for in the stages when Jannsens supposed crossing-over to occur the chromosomes are rather loosely twisted, so that loops of very small length do not occur as often as longer ones (thus, very near one point of crossing-over the strands seldom cross back again). I therefore determined the mathematical relations which would exist between crossing-over frequencies, if crossings-over had a chance distribution with reference to one another, in order to compare these figures with those obtained by experiment. On the assumption that separation between A and B has no influence on separation between B and C, if crossing-over occurs between A and B in 10 per cent. of cases and between B and C in 20 per cent. of cases, then, among those ten cases in a hundred where crossing-over between A and B occurs, 20 per cent. (*i. e.*, 2 cases) would be cross-overs between B and C as well; in other words, the per cent. of double cross-overs would be equal to the product of per cents. AB and BC (formula 1). The easiest way to determine the correctness of the assumption in any given case, therefore, is to compare the observed per cent. of double cross-overs with per cent. AB \times per cent. BC.

Another relation besides was found to hold between the theoretical linkage values, dependent upon the relation in formula 1. For it is easily seen that the number of *separations* between A and C must always be equal

to the sum of the number of crossings-over between A and B, and between B and C, minus all those crossings-over contained in the cases where coincidence occurred, and in which A and C, therefore, failed to separate,—*i. e.*, minus twice the number of *cases* of double crossing-over. Hence, if formula 1 is correct, then it must also be true that per cent. AC = per cent. AB + per cent. BC — 2 (per cent. AB × per cent. BC) (formula 2). This formula was originally expressed not only in the above terms, where the “per cent. of separations” (*i. e.*, ratio of separations to the total number of cases) is used as the index of separation frequency, but also in terms of the so-called “gametic ratio”—the ratio of cases of non-separation to those of separation—for this was the way of indicating degree of linkage then used by all investigators of the subject. The latter index gives much more complicated formulas, however, so the writer pointed out at the same time that per cent. of separations would afford a much more useful measure of linkage.

Later, Trow also worked out and published the same formula (no. 2)—in terms of the “gametic ratio”—and it is generally known as “Trow’s special hypothesis” (18). But on the reduplication hypothesis held by Trow, and by the other English geneticists who do not accept the chromosome explanation, the formula would be supposed to result, not from the fact that crossing-over between A and B was independent of that between B and C, but from the fact that “reduplications” AB and BC were independent, not being disturbed by any “primary reduplication” AC. Adherents of the reduplication hypothesis have been much concerned as to whether or not their results confirmed the assumptions made in Trow’s formula, and have in one or two instances calculated that they did. Let us examine for a moment the requisites for proving such a conclusion. As above shown, the whole matter turns on the frequency of coincidence of separations AB and BC (*i. e.*, on the frequency of “double crossing-over”) and the question can be settled by determining directly the amount of this coincidence.

If the per cent. of double cross-overs = per cent. $AB \times$ per cent. BC (formula 1), then the assumption that separation frequencies AB and BC are independent is correct. As offspring from a back-cross all show what factors they received from the hybrid parent, a back-cross involving the three factors A , B , and C at the same time will answer the question at once, for all the cases of coincident separation (double cross-overs) that occur can be counted. But where the hybrids, instead of being back-crossed, are inbred—a practice followed by adherents of the reduplication hypothesis—then it is impossible to tell which F_2 individuals come from gametes of the classes which we may term double cross-overs, unless one of these classes is the triple recessive, and then the only double crossovers which can be known as such are those very rare individuals that happen to result from the union of two double crossover gametes. The British workers have, therefore, not been able to find the proportion of double cross-overs directly, to compare this with formula 1, but have tried to determine the frequency of coincidence indirectly, by using the method followed in formula 2. That is, they determined the relations existing between frequencies AC , AB , and BC , as calculated from their F_2 counts, for, as above shown, the greater the frequency of double crossing-over, the more will AC be cut down in proportion to AB and BC . And it seemed evident that, if the relation of AC to AB and BC was just that given by Trow's formula ("2"), then coincidence of separations must have the frequency demanded on the assumption that separations (or "reduplications") AB and BC occur independently of one another. As a matter of fact, however, this method offers no answer to the question, unless almost impossibly large F_2 counts are obtained, for otherwise *the independent random fluctuations of these three values in this kind of count are so great that any deviation in AC due to excess or deficiency of double crossing-over would be quite lost to sight.*

The question was, however, immediately and definitively answered in *Drosophila*, before Trow's paper ap-

peared, by examination of Sturtevant's extensive backcrosses, especially of those involving three pairs of factors at once. As the results did not conform to the formula, it was not published, but as Trow has since raised this question publicly and the adherents of the reduplication hypothesis are still discussing it, it may not be out of place to have given an analysis of it here, and to recall the fact that it had already been tried and rejected. Besides, as will appear below, a discussion of the relations which would exist if crossings-over were independent of one another is a necessary preliminary for a treatment of the relations which do exist between linkage values.

The results showed that double crossing-over does not, as a rule, occur as frequently as would be expected if, as the above formulæ assumed, it were purely a matter of chance whether or not two cross-overs happen coincidentally. In a sense, then, the occurrence of one crossing-over interferes with the coincident occurrence of another crossing-over in the same pair of chromosomes, and I have accordingly termed this phenomenon "*interference*." The amount of interference is determined by comparing the actual per cent. of double cross-overs with the per cent. expected if crossings-over were independent, *i. e.*, if they had a purely chance distribution with reference to each other. Now, the per cent. which would occur on the latter expectation has already been given by formula 1 as per cent. $AB \times$ per cent. BC . If, then, the observed per cent. of double cross-overs were divided by per cent. $AB \times$ per cent. BC , we would obtain a fraction showing what proportion of the coincidences which would have happened on pure chance really took place. This ratio of observed double cross-overs to the chance expectation appears to me to furnish the most useful measure of interference. The ratio is itself best expressed in per cent., and it may be called the relative coincidence, or simply "coincidence." If the "coincidence" is low, this means that there has been much interference, for most of the double cross-overs expected on chance were prevented from appearing; conversely, if coincidence is high, the

interference must have been very weak. Some illustrations may make the meaning of this index clearer. If, for example, coincidence is 0 per cent. no double crossing-over is occurring; the interference between one crossing-over and another is then complete. If coincidence is 45 per cent., this figure does not mean that 45 per cent. of the individuals are double crossovers, but that 45 per cent. of the number of double crossovers which would be expected as a result of pure chance (whatever that number may have been) actually appeared, 55 per cent. having been "interfered with," or somehow prevented from occurring. If coincidence is 100 per cent., there has been no interference, for the same number of double crossovers appeared as expected on the ground that the two crossings-over did not interfere with each other's occurrence. 110 per cent. would mean that if one crossing-over occurred, the other was 10 per cent. *more* likely to occur than in cases of random distributions of crossings-over. This would be "negative interference," for as coincidence increases interference decreases.

On Janssens's theory that crossing-over takes place in the strepsinema stage, when the chromosomes are twisted in loose loops, crossing-over would very seldom take place at two points very near together, for this would require a tight twisting of the chromosomes. Accordingly, on this theory interference was to be expected; furthermore it would be expected that interference was very great between crossings-over that were in neighboring regions; but between crossings-over further apart there should be little or no interference. The results were according to this expectation; they indicated strongly that the interference was very great for crossings-over short distances apart, but progressively diminished as the distances considered became greater. The conclusion drawn was that crossing-over took place as postulated on Janssens's theory, when the strands were loosely twisted in strepsinema, although the twisting and crossing-over did not take place in the stereotyped manner suggested as a first possibility, in the earlier part of this section. For there

was evidence that the distances between the two points of crossing-over in double cross-overs were variable; but this again corresponded with the fact that the chromosomes of *Batrachoseps* and other forms, as seen under the microscope, did not always twist in loops of the same length. Furthermore, if it be supposed that in most maturing eggs of the fly the homologous chromosomes twist tightly enough to cross at least once or twice, as is certainly the case in *Batrachoseps* and many other forms, it must be concluded that at not every point of crossing does actual "crossing-over" (recombination of strands) take place, for it was found that nearly half of the factor-groups emerged without having undergone any crossing-over at all. And this, in turn, corresponded with the observations of Janssens and others, which showed that at some at least of the points of crossing of homologous chromosomes, the latter merely untwisted again without having undergone the "chiasmatype" process. Here, then, was a theory of crossing-over that seemed complete, so far as connecting the genetic facts with the cytological observations was concerned.

B. Possible Mechanisms of Crossing-Over

There is one very unsatisfying point, however, in this original scheme of crossing-over. That is, it postulates that crossing-over occurs at a comparatively late stage in synapsis, when the strands have become very much shorter and thicker than the long delicate threads which first came into contact with their homologues (see Fig. 6). Now, in crossing-over the chromosomes must come into contact, and break, at *precisely* homologous points, otherwise factors would be lost or gained by them when crossing-over occurs. But presumably the factors are set very close together in the line, judging by the fact that mutations in new "loci" (positions in the chromosomes) are still as numerous as ever, and that, if the whole chromosome is packed with factors as close together as, judging by their linkage relations, they seem to be at certain places in it, it must contain at the very least 200 factors. It is

difficult to conceive how this cleavage of ultramicroscopic nicety can take place properly at a stage when the chromosomes are so coarse and short. The observations of Vejdovsky and others, taken in connection with the genetic results from *Drosophila*, render it practically certain that the factors are really disposed in an extremely fine,

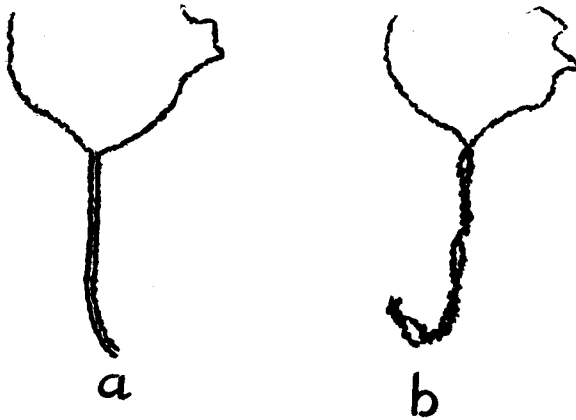


FIG. 6. Chromosomes during an early stage of synapsis (amphitene). In some preparations the apposed threads seem parallel, as in *a*; in others they seem twisted about each other, as in *b*.

long thread or "chromonema," which, during the metaphase and anaphase of mitosis, is coiled up very closely in more or less spiral fashion (probably within a viscous sheath of some sort), to form the thick dense chromosomes, but which, in the resting period and during the early stages of synapsis, becomes, to some extent at least, uncoiled and drawn out again. In this state, then, the chromosomes first pair, as shown in Fig. 6. Thus precisely homologous parts of the frail threads may become apposed to each other, so that this stage, which is called the "amphitene" stage, would seem to be the one best "adapted" for the occurrence of crossing-over. Later, when each chromosome becomes, presumably, a thick spiral, there would seem to be much greater mechanical difficulties in the way of exact apposition and breakage of parts.

On any possible theory of crossing-over, however, the known facts concerning interference should be capable of

interpretation. If crossing-over occurred during the "amphitene" stage, or not long after, would there be any possible explanation of the fact that one point of crossing-over is generally far removed from another? The explanation might be found simply in the fact that each of the "leptotene" chromosomes—*i. e.*, the finely drawn out chromosomes which are just about to undergo synapsis—pursued a general course that had few close turns in it. (For possibly it maintains the same general direction as it had when it was short and thick; the reader will recall that Boveri found that chromosomes preserve their approximate shape and position from one cell division to the next.) When, therefore, the leptotene chromosomes are being brought together by the synaptic attraction which homologous loci then bear for each other, the threads are usually crossed only at a few points, and these are generally far apart. If these initial points of crossing—which, it will be observed, have been determined by the original positions of the threads, and not by any twisting—are the points of crossing-over, interference would be accounted for, and would, in effect, be of the same general nature as on the mechanism of crossing-over postulated by Janssens.

On this second scheme of crossing-over (Fig. 7), it might at first seem hard to see why recombination—*i. e.*, "crossing-over"—should occur where the threads cross, but it should be remembered that the two threads, while coming together, often lie in about the same plane both above and below the point of crossing. If they keep to this original plane as they draw together, they will come to have the same plane of apposition just above and just below the crossing point,—although the sides of the filaments that face each other will be just the opposite in the two places; consequently, the threads at the crossing point must undergo a very sharp twist, and if, as we must suppose, they are somewhat viscous, this may result in their breakage and recombination, or, perhaps, first in their fusion, and, later, when the pieces of the same chromosome above and below the point of crossing are wrenched

apart in opposite directions by mutual repulsion of the strands or by pulling of spindle fibres, in breakage of parts originally together. (So perhaps fusion might occur during the amphitene and breakage in the strepsinema stage; this would be a combination of schemes 1 and 2 which would account both for the exact apposition of parts and for the phenomena observed by Jannsens.) Be this as it may, at any rate, the negative argument may be given that it is just as hard to account for recombination at a later stage in synapsis as at this stage, even overlooking the objection of the thickness of the threads.

There is a serious objection to the scheme just given, however, in that, as the threads come together, they seem, in many preparations, not to keep their original plane of apposition, but to twist tightly about each other, like the strands of a rope, throughout their entire length (see Fig. 6). It is possible that the twisting of one thread about the other is merely apparent, however, and that the threads lie parallel but are simply coiling up in a spiral, in the process of forming the shorter, thicker prophase chromosomes; for, unless the spiral were very delicately preserved by the fixing agent, there would be apparent knots in it as though there were a twisting of two strands about each other. Moreover, there is evidence indicating that this tight twisting occurs only in certain species of animals. But let us assume for the moment that this very tight twisting really takes place during the amphitene stage in flies, and that crossing-over takes place at this period (this we may call scheme of crossing-over number three). Would there then be any way of explaining why one crossing-over should interfere with another near by, in view of the fact that the loops are of such small dimensions? In seeking an answer to this question, it will be helpful to bear in mind that crossing-over can be divided into just three essential processes—a bending of the chromosomes across each other, a breaking of the threads, and then a fusion of adjoining pieces (or, perhaps, the fusion of the homologous chromosomes comes first, and then the breaking of the original chromosomes at that

point). It follows from this that interference must in any case be due to one of the following three general causes: (1) Either the chromosomes are not likely to bend across each other twice at points near together (*i. e.*, the loop tends to be long), or (2) breakage at one point for some reason interferes with another breakage nearby (even though the threads are crossed at both of these points), or (3) fusion of chromosomes at one point in some way interferes with fusion of threads which are crossed in a neighboring region. That fusion at one point could interfere with fusion at another point can scarcely be imagined. And if crossing-over occurs according to scheme number three, the "loop explanation" must also be thrown out. Consequently, if crossing-over occurs at a stage of tight twisting the breakage of the threads at one point must somehow be considered to prevent another break near by. In explanation of this, breakage might be thought of as resulting from the tightness of the twisting, for then a breakage of the threads at one point would relieve the tension of the filaments for some distance along the line and so tend to prevent another breakage from occurring near by. (Later, when threads reunited at the point of breakage, pieces from homologous chromosomes would be as apt, or more apt, to lie end to end, and therefore to join, than pieces of the same chromosome. As a partial explanation of why the fragments should join again at all, it might be supposed that only the chromonemas break, the fused sheath which envelops the pair still holding the pieces together.)

It is fully realized that the above discussion is highly speculative. It is intended, however, not as a presentation of conclusions, but as a tentative suggestion of possibilities, in order to obtain some system of ideas that may furnish a temporary basis for a real attack—experimental and observational—upon the subject.

Tests for These Alternatives

Is there any way of obtaining evidence as to which of these three schemes of crossing-over is the more probable

one? Light might perhaps be thrown on the question by a closer study of interference, and it was largely for this reason that the experiment described in section V was undertaken. If, for example, interference was a result of length of loop (as would be true in schemes I and II), and the length of the loop tended to vary more or less in both directions, about a given mode, then coincidence would be relatively higher between crossings-over which were that distance apart, than between crossings-over nearer together or still further apart. In other words, as may be seen from Fig. 8, for small distances, the relative

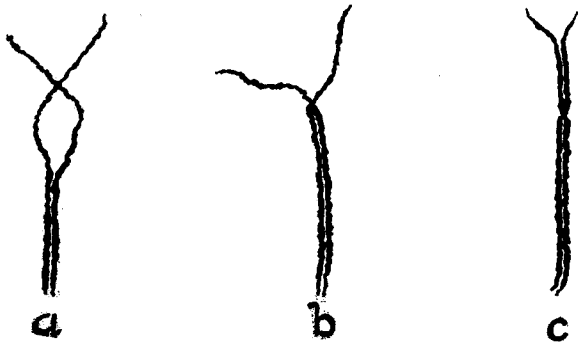


FIG. 7. Diagram to illustrate the second scheme suggested for crossing-over. The amphitene threads become sharply crossed at a particular point.

coincidence would be very small (interference high), for longer distances much greater, and with still longer distances coincidence would fall again (interference would rise). For distances double or triple the length of the loop—if the chromosomes were as long as that—coincidence would rise once more. Secondly, on the “loop explanation” of interference just outlined, coincidence should, at the modal distance, rise above the 100 per cent. level, for crossing-over would occur at a given point (K) *more* often in those cases when there is crossing-over at another point (I) lying at the modal distance from K, than in the average case. Of course it might be, however, that there was no modal length of loop—that although short loops were infrequent, all loops above a certain size were equally frequent, or that the longer the loop, the more

frequent it tended to be. In the former case coincidence would rise to a certain level, as distance between the points of crossing-over considered increased, and would after that remain constant; in the latter case it would rise progressively, and might or might not reach or pass the 100 per cent. level.

On the other hand, if crossing-over is due to a breakage of tightly twisted threads, not so many different kinds of variation of coincidence, with increase in distance, would be theoretically possible, but a condition something like the one last mentioned must always obtain. For, on scheme 3, the interference of a breakage with the tightness of twisting and consequent chance for another breakage must decrease progressively at greater and greater distances from that breakage; coincidence would thus rise until finally it reached the 100 per cent. level expected on chance. It would never rise much² beyond this, as one break could never make another *more* likely to occur; neither could coincidence fall once more, with a still greater distance (as it could on the loop scheme, after a "modal distance" had been reached). If, therefore, it should be found that, for certain (modal) distances between two points of crossing-over, coincidence ran well above 100 per cent., or that, beyond certain distances, coincidence fell again, there would be good evidence that crossing-over did not occur at a stage of tight twisting. If, on the contrary, it were found that crossing-over coincidence rose progressively with distance, until it reached the 100 per cent. mark, but neither went much² beyond

² Even on scheme III, coincidence could finally rise slightly above 100 per cent., for although one break (I) could not help another (K) to occur, no matter how far away the latter (K) might be, still it might, by preventing the occurrence of other breaks (J), in between these two, give more chance for the occurrence of the break farther off (K), since in this way the interference of breaks J with K (which is stronger than the interference of the more distant I with K) is removed. Thus break K might occur more often when I also occurs than in the average case, and so coincidence would rise above 100 per cent. However, it would be easy to distinguish between the slight rise in coincidence above 100 per cent., due to this cause, and the rise which would exist on the loop explanation of interference if I and K were separated by a distance about equal to the modal length. For, in the first case, *considering only gametes in which no crossing-over at all took place in between*

this, nor fell again later, and if cytological measurements should then substantiate the judgment, based on inspection, that the loops did have a modal length during the strepsinema stage, there would be good evidence that crossing-over must occur at an early stage of synapsis.

Other peculiarities of coincidence also might be found which would permit of explanation on one scheme and not on another. In groups II and III, for example, there seem to be peculiarities in the coincidence relations in cases where the chromosomes differ in regard to the factor C, or a similar factor. And a comparison of coincidence in different regions of the chromosome in any given case or in the same region of the chromosome in cases of linkage variation, might very well reveal relations that lend evidence to one scheme of crossing-over or another. Even a determination, not of coincidence, but merely of linkage variation itself, in different parts of the chromosome, might in some way shed light on the subject. In the case of the third chromosome, experiments of this sort are now under way with multiple stocks which I have made up for this purpose, and Sturtevant is conducting similar experiments with group II. The first requirement, however, is obviously an accurate study of normal coincidence, and it therefore became necessary to determine the coincidence for points various distances apart, preferably in the same experiment. But to work with a great many factors in a group at once introduced new difficulties, which made special methods necessary, as will be explained later. Before considering this experiment, it will be desirable to consider other lines of evidence and modes of attacking the problem of crossing-over.

The cytological evidence which Janssens presents for crossing-over is entirely directed towards proving that crossing-over occurs during strepsinema or later. In strepsinema the chromosomes, as already mentioned,

(*at J*), it is easily seen that the proportion of breaks at K would be lower when breakage occurred at I than when there was no breakage at I, whereas in the second case, the proportion of breaks at K would in such gametes be higher when there was breakage at I than when there was no breakage at I.

become much shorter and thicker than in the amphitene stage, and each chromosome in the pair can in many preparations be seen to have split lengthwise, *i. e.*, the "tetrads" have formed preparatory to the two maturation divisions. Janssens often finds the four threads placed somewhat as shown in Fig. 9*a*, two of the threads crossing at one or two points, but otherwise being rather widely separated, and the other two threads rarely crossing but lying close to whichever one of the two threads first mentioned happens to be on the same side, and merely bending inwards and then back again where the first two threads cross. The peculiar crossing of two of the threads and the bend in the other two, as shown at point L, he interprets, in the way shown in Fig. 9*b*, as meaning

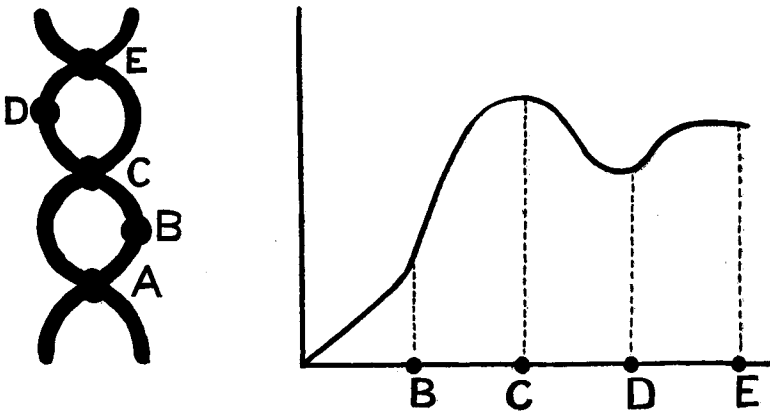


FIG. 8. Diagram to show possible coincidence relations on schemes I and II. The chromosomes are represented as crossing-over at A, and twisting in loops of the most usual (modal) length. It will be seen that a crossing-over at A will rarely coincide with one nearby—at B—since then the chromosomes would have to twist in loops much smaller than the modal length. But it will often coincide with one at C, seldom with one at D, and often again with one at E. The *relative coincidence* of crossings-over at various points on this chromosome with crossing-over at A is shown in the curve on the right.

that both pairs of threads originally were twisted across each other, but that the two homologous threads which were originally on the inner side, and so touched each other, underwent recombination, *i. e.*, "crossed over," at the point of contact; each of the new chromosomes thus formed, therefore, would lie entirely on one side or the

other; the other two threads, on the contrary, are supposed not to have undergone recombination ("crossing-over") and therefore would still lie across each other.

It would seem equally possible, however, to interpret these figures as meaning that (as shown in Fig. 9c and 9d) when the four threads began to separate into two pairs, separation happened to start at some points (A and C) between the identical halves and at other points (B) between the homologous chromosomes, it being merely a matter of chance in which way the separation started to take place. It will be seen that this would result in the formation of just such cross-figures, between two regions where separation took place in opposite ways, as Janssens finds.

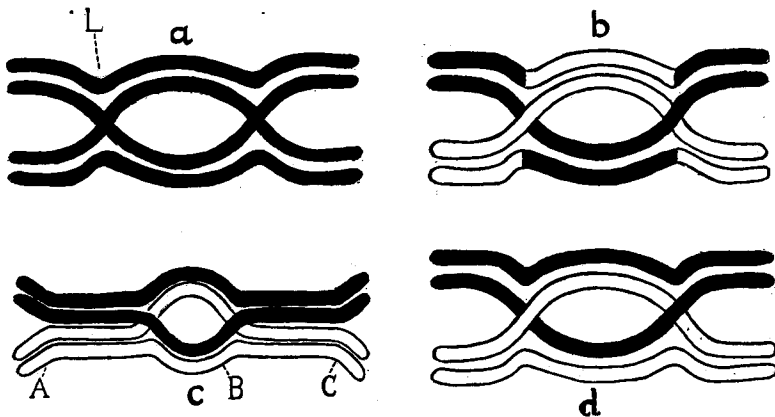


FIG. 9. (a) The chiasmatype described by Janssens. (b) His interpretation of it. (c) and (d) A suggested alternative interpretation.

Another point in Janssens's evidence is that the prophase chromosomes of maturation divisions not only show the strands crossing, at points, but often bending in towards each other near the middle, as though they had formerly crossed there, and later undergone crossing-over. It would seem possible, however, that this figure is merely due to the chromosomes remaining in contact more closely at the point where the spindle fiber is attached, and spreading apart elsewhere,—a relation which figures of Bridges and others show to exist between the two identical halves of chromosomes in the prophases of oogonial mitoses.

Finally, Janssens says that crossing-over is indicated by the fact that the chromosomes often seem to have sunken into one another at the crossing point. This detail, which would be very difficult to establish, might, of course (if it had any significance at all), merely mean that the chromosomes were still closely attached at the point where they had *previously* crossed-over. It seems incautious, therefore, to regard the cytological evidence as showing more than the possible means of the crossing-over which the evidence from factor and chromosome distribution demonstrates to occur.

Some of the Orthopteran material which gives such clear-cut chromosome figures might perhaps settle the point whether crossing-over occurs at the stage of four threads, as Janssens believed. For it is reported by Wenrich (19) that homologous chromosomes can sometimes be distinguished from one another in prophases by differences in the size or shape of contained granules, that are constant for the particular individual. Moreover, the four threads are clearly distinguishable in the prophase of the first maturation division. If a female could be found (there is some reason to believe that crossing-over does not occur in the male) which showed a difference in respect to two granules at different points in the same pair of chromosomes, then, if Janssens's theory is right, it would happen that, in some of the oocytes, of the four post-synaptic threads two would have a new combination of granules and the other two would not show any interchange. But on the view that crossing-over occurs earlier—the identical halves being formed after interchange has taken place—all four threads would be of a new combination in those cases where crossing-over of chromosomes in the region between the two pairs of granules had occurred at all.

There is an essentially similar, although much less certain, genetic test possible. For if two threads may cross over and not the other two, then, if non-disjunction of X-chromosomes should occur in that maturation division

when the threads that crossed would normally have separated from those that did not, the egg would come to contain two X-chromosomes, one of which was a cross-over but not the other. In the usual type of non-disjunction, the X's never cross over—presumably because they paired with the Y (which was present in these cases as an extra chromosome), so this type of non-disjunction could not afford a test of the theory. But it is to be expected that non-disjunction should sometimes occur by mere accident without the interference of a Y, and since in these cases the X's could have crossed over, such cases of non-disjunction might furnish a test of Janssens's theory. In 1913, in an experiment designed for this purpose, I obtained a fly which had received two maternal X-chromosomes by reason of non-disjunction in its mother, and in which one of these X-chromosomes proved to be a cross-over but not the other! The fly resulted from a cross of a female which contained in one X-chromosome bifid and vermilion, and in the other chromosome eosin and bar, by a normal male. It itself contained in one of its chromosomes bifid and vermilion, and in the other bi, v and also bar. Since then Bridges has obtained other exceptions of the same general sort. But on further consideration it appears that this result really proves nothing, for the non-disjunction may just as well have taken place in an oogonial division. In this way an oocyte would result that contained three X-chromosomes. At synapsis two of these could cross over with one another, and the egg could then receive a cross-over chromosome and also an X that had not crossed over. To prove that the non-disjunction was not of this type, but really occurred in a maturation division, *i. e.*, that the two threads originated from one tetrad, it was then thought necessary to obtain flies in which both of the X-chromosomes received by non-disjunction had crossed over, but each at a different point (or one of them at two points).

In a case of the latter sort the fact that both chromosomes had crossed over at some point might be taken as meaning that both had been in the synaptic tetrad, and so

that the non-disjunction had occurred in the maturation division in the mother fly, or else that both were derived from the two halves of a single (cross-over) X-chromosome which underwent non-disjunction in an embryonic cell division of the individual itself. But the fact that the two chromosomes are not identical would rule out the second possibility. The result thus might seem to show that in the same tetrad one strand may have crossed over at a certain point and not another strand, there being four threads present when crossing-over occurred. But suppose in some oogonial division the halves of neither X disjoin, so that an oocyte gets four X's. Two X pairs will form and an egg may thus get two X's that crossed over differently even if crossing-over is at a 2-thread stage.

Since first printing of this paper, one such exception has been found, by Bridges; he has besides gotten some exceptions of the first type, that may be explained by non-disjunction of one X in an oogonial division (since one X had crossed over—but not the other), and also one other exception, which had received two similar double cross-over chromosomes. The latter peculiar circumstance must have resulted either from a non-disjunction, at the maturation division in the mother, of two strands of a tetrad, both of which had crossed over in the *same two* places, or from a non-disjunction, in an embryonic cell division of the individual itself, of the two halves of the single (double cross-over) X-chromosome, which, on this view, was originally present. But the latter explanation is very improbable, for, unless the non-disjunction occurred in the first cleavage, only a small part of the fly would be composed of cells descended from the one into which the 2 X's entered; most of the cells, therefore, would contain only one X and these would necessarily be male; thus the fly would be a gynandromorph. Moreover, all the cells derived from the one which, in the non-disjunctive division, failed to receive either half of the X-chromosome, would probably die. Hence the evidence is fairly good that in this case the two double cross-over X-chromosomes represent two strands of a tetrad. Since

these two strands, although both double cross-overs, were both just alike, we must conclude either that they were both derived from the same strand, after it had already crossed over—in which case crossing-over must occur at a stage in synapsis before the homologous chromosomes split to form tetrads—or else that the tetrads were formed first, and that then crossing-over occurred at two points coincidentally in the case of both pairs of threads, and at identical points in both. It is not probable, however, that, if crossing-over occurs at the stage of four threads, these two pairs of threads would both cross over at the same points, for according to the observations on which Jannsens bases the idea that crossing-over occurs at this stage, a crossing-over of both pairs of threads at the same place rarely happens. The evidence thus far gained from non-disjunction is, therefore, rather in support of the theory that crossing-over occurs at an early stage in synapsis.

D. A Case of Crossing-Over in an Embryonic Cell

It may not be out of place here to record an exceptional case of crossing-over in the male, which has not been explained. No other case of crossing-over has hitherto been found in the male *Drosophila*. It had been established by Altenburg and the author that the factor causing truncate wings is in the second chromosome, and further that the truncate factor is dominant under certain conditions, but it does not usually express itself unless certain intensifying factors—one in the first chromosome and one in the third—are present; even then, the character sometimes fails to develop. Thus, if a hybrid truncate male is produced by a cross of a truncate female to a black pink male (black is in chromosome II and pink in III), when this hybrid is back-crossed again to black pink females, only the gray flies will carry the factor for truncate, since in the male truncate can not cross over with the black in the homologous second chromosome. But few of the gray flies from such a cross except the gray, red-eyed females will show the truncate character, for the others will not

contain both of the intensifying factors; and even in the gray red females the character will not always develop.

A typical count for such a cross was as follows:

Gray Red			Gray Pink			Black Red			Black Pink		
Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.
57	23	7	2	14	64	0	0	74	0	0	82
2	29	47	1	8	62	0	0	67	0	0	73

(The count of females is shown on the upper line, the count of males on the lower.) A brother of the above male, however, when similarly back-crossed, gave the following count:

Gray Red			Gray Pink			Black Red			Black Pink		
Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.
0	0	32	0	0	19	16	20	1	0	18	7
0	0	15	0	0	23	0	6	11	0	3	22

The sex-linked intensifier and the third chromosome intensifier are inherited normally as before, for the females have wings much more truncated than the males and the reds are more truncated than the pinks. But, although the truncate parent of this male contained gray in the same chromosome as truncate, and the long-winged parent contained black with long, all the truncate has crossed over, away from the gray factor and into the chromosome with black! Not a single fly has the old combination, gray truncate. It is next to impossible to imagine that the chromosomes of the second pair crossed over in the synapsis period of all the spermatocytes, and in all of them, between just these particular loci, when normally there is no crossing-over at all in the male and only 30 per cent. of crossing-over between these loci even in the female. It is, therefore, necessary to conclude that crossing-over took place once for all in a cell of the embryo, and that, as usual, it did not occur at all during spermatogenesis, although all the spermatocytes, of course, inherited the cross-over combination. It is impossible to tell whether or not the chromosomes underwent the regular process of synapsis at this early stage,

and whether they crossed over when long drawn out or when short and thick, but at least the fact remains that crossing-over may, in abnormal cases, take place in a cell before the definitive growth period is reached, and even in an individual (*Drosophila* male) in which no crossing-over is the established rule. This fact is not utterly surprising, inasmuch as even in somatic and gonial cells of Diptera homologous chromosomes show a marked tendency to lie near together (*i. e.*, to attract each other), and in Metz's preparations they may not infrequently be found even twisted about each other somewhat.

The fact that crossing-over occurs only in the female *Drosophila* is naturally of great interest, although it is of unknown significance. In the silkworms, on the other hand, Tanaka has discovered that crossing-over takes place in the male, but not in the female. Curiously enough, although these seem at first sight to be opposite cases, in both it is true that crossing-over takes place in the homozygous sex, but not in the heterozygous, for in *Drosophila* the female is homozygous for sex, the male heterozygous, and in the moth these relations are reversed. Recently, however, Castle and Wright have published data for the rat which, if sufficiently extensive, show that crossing-over happens in both sexes. The plants in which crossing-over has so far been studied have all been hermaphrodites, and crossing-over takes place in both their spermatogenesis and oogenesis. There is, therefore, at present no general rule which can be stated, in regard to which sex crossing-over occurs in. This fact should be taken into account in weighing the cytological evidence in regard to crossing-over, obtained in forms in which the occurrence of crossing-over has not been studied genetically. For in such cases there is always the possibility that the cytological studies are being conducted on individuals in which crossing-over does not occur and which would consequently give results quite irrelevant to the subject.