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CHIASMATYPE AND CROSSING OVER

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Two short papers by Janssens, published in the *Comptes Rendus* of the *Société de Biologie* for April and May, 1919, outline an interpretation of the maturation-phenomena in Orthoptera in agreement with his earlier chiasmatype-theory ('09) based on the corresponding phenomena observed in urodeles. It is a matter of so much importance that all phases of this question be fully discussed that we venture to report and examine the conclusions announced in these new communications. For this purpose we have found it convenient to divide the discussion into two parts, one dealing with the matter more from the standpoint of strictly cytological observation, the other more from that of the possibilities suggested by genetic analysis. In order to avoid repetition we have numbered the figures consecutively, but each author is responsible for the part under his name.

I

A. CYTOLOGICAL VIEW OF THE CHIASMATYPE THEORY

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Professor Janssens's results are as yet illustrated only by diagrams, which leave us in doubt concerning some very important details; nevertheless, a cytologist may be permitted to indicate at this time how the conclusions are related to those of other cytologists who have ex-

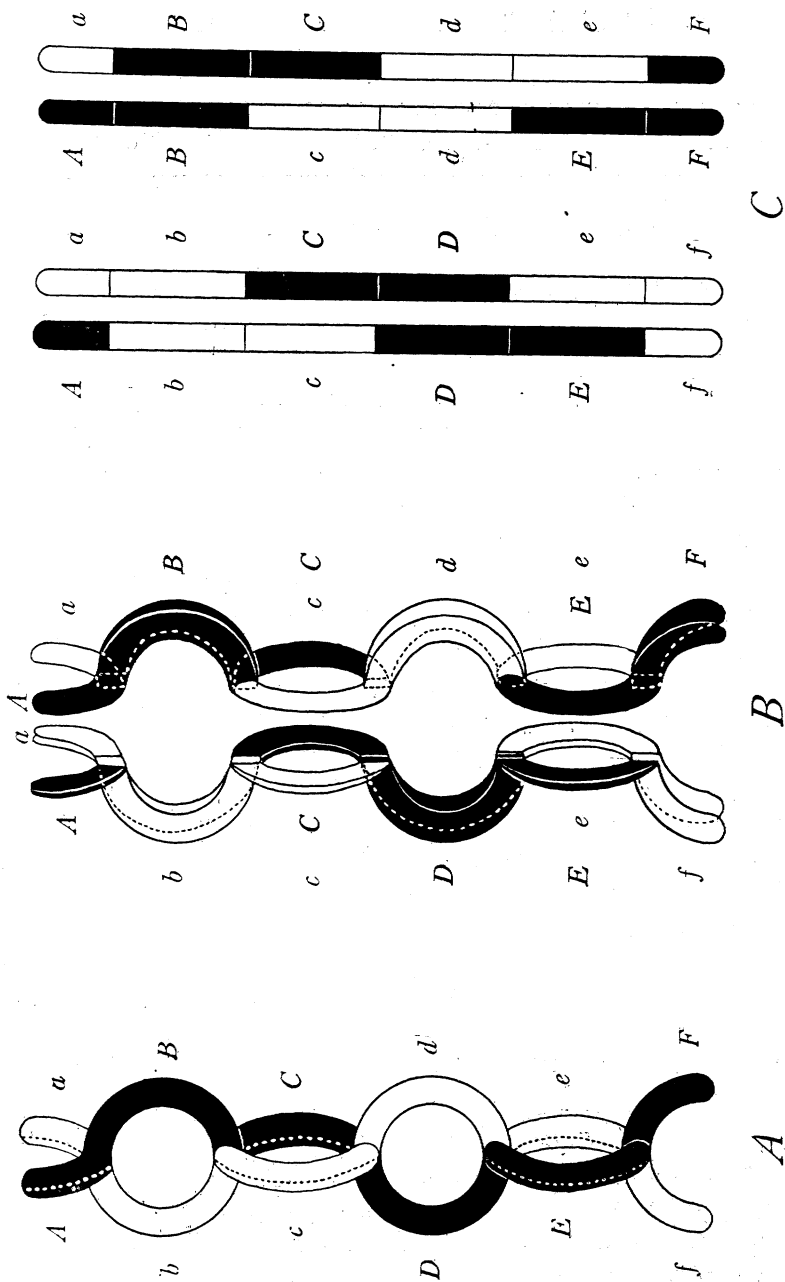


FIG. 1. Janssens's interpretation of the compound rings in Orthoptera (Janssens). In each case the synaptic mates or allelomorphs are in black and white respectively. Corresponding or homologous regions of these mates are designated as A and a, B and b, etc. A, the ring-series in perspective; B, its first division (the second division is at right angles to this, nearly in the plane of the paper); C, the four resulting classes of chromatids.

amined the phenomena in Orthoptera and other insects. Incidentally I may remark that Janssens cites from one of my own papers ('12) in support of his general theory and also copies from another ('13) a series of general diagrams by which the theory was illustrated. As he

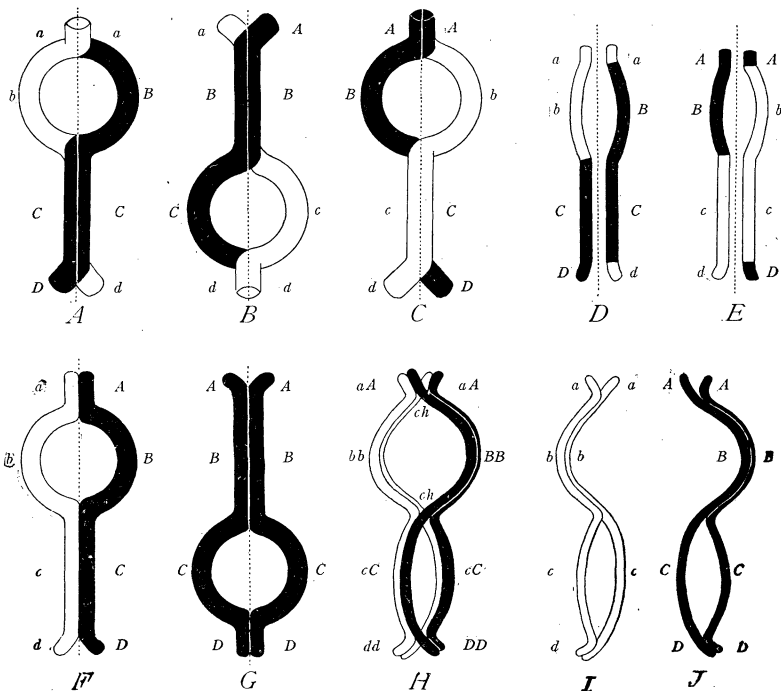


FIG. 2. History of the double rings (A-E, after Janssens). In each case the synaptic mates are black and white, respectively, corresponding regions marked *Aa*, *Bb*, etc., as in Fig. 1. A-E show Janssens's interpretation of their history. A, double ring from one side; B, the same rotated 90° to the left; C, the same after rotation through 180°; the dotted line shows plane of first division; D, E, the four resulting classes of chromatids; F-H, corresponding figures showing the composition of these rings as heretofore described; I and J, the two resulting classes of chromatids, with no cross-overs.

points out, these diagrams were too much simplified to give an adequate representation of his views; but a critical account of cytological *minutiae* was obviously inexpedient in a presentation intended only to make clear to a general audience the nature of Janssens's fundamental assumption. I am glad however to see from these latest

papers that he does not consider the diagrams to have misrepresented the gist of the matter.

So far as can now be judged, Janssens's latest studies add nothing to his paper of 1909 that is new in principle. They are in the main an elaboration of his earlier conclusions concerning the double-ring and double-cross types of tetrads, which were illustrated in his former work by diagrams XI, XVI, XIX and XX. These two forms of tetrads are closely related, and each of them shows in the prophases of meiosis a two-strand chiasma—*i. e.*, two threads which seem to pass over from one synaptic mate to the other, crossing each other midway between them, as in Fig. 5, I or 5, III—such as formed the main basis of the original chiasmatype theory. It may be needless to describe these tetrads, which are perfectly familiar to cytologists, but for the sake of clearness I will briefly review their composition as now generally understood.

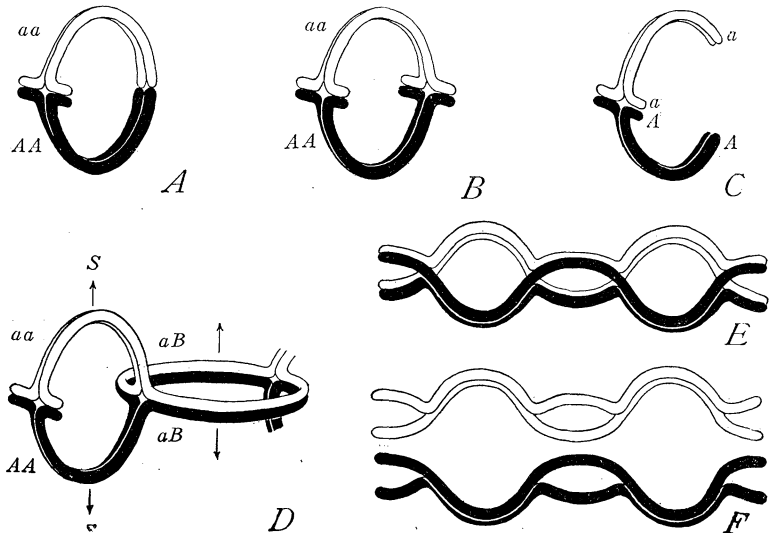


FIG. 3. Diagram to show relation between the single rings, double crosses, double ring and multiple ring types of tetrads. In each case the synaptic mates are black and white respectively. *A*, single ring with one pair of lateral arms; *B*, same with two pairs of arms; *C*, double cross type with curved arms; *D*, double ring, showing spindle-attachments (*S, S*); *E*, multiple ring type (viewed in somewhat different perspective); *F*, mode of division of such a tetrad.

Ring-tetrads may be single, or may consist of two or more rings joined together in such a manner as to be successively at right angles to one another, as is schematically shown in Janssens's diagram, here reproduced in Figs. 1 and 2 *A-C*. Single rings of the type here in question (Fig. 3 *A, B*) were I think first clearly described and figured in my laboratory by Paulmier ('98) in Hemiptera, though he did not correctly make out their mode of origin. Similar rings were subsequently studied in many other animals, *e. g.*, in Orthoptera by McClung, Sutton, Granata and others, in urodeles by Janssens, and in annelids by the Schreiners, Foot and Strobell and others. More recently they have been carefully examined by a number of observers, in particular by McClung ('14), Robertson ('16), and Wenrich ('16, '17). The single ring-tetrad (Fig. 3 *A, B*) consists of a more or less open ring, split lengthwise into two closely apposed halves and cut crosswise at opposite points by two sutures which divide the ring into two semicircular half-rings. The latter are now regarded by practically all observers (Janssens included) as the synaptic mates, joined by their ends but elsewhere widely separated so as to lie on opposite sides of the ring-opening, and each longitudinally split. The longitudinal cleft lies therefore in the plane of the future equator-division, the cross-sutures in that of the reduction-division. At one of the cross-sutures, less often at both, the longitudinal halves of both synaptic mates are commonly drawn out at right angles to the ring (in the manner made clear by Fig. 3 *A, B*) thus forming two lateral arms, each longitudinally double, so that this part of the ring, as seen in face view, offers the figure of a double cross. If the ring be supposed to break in two at the opposite suture and the half-rings to straighten out completely it would become a simple double cross-figure with two short arms and two long (Fig. 3 *C* or 5 III). If, on the other hand, the lateral arms of a closed ring be supposed to elongate still more and to bend away from the original ring until they meet,

they would give rise to a second ring continuous with the first but at right angles to it, as shown in perspective by Fig. 3 *D*; and by repetition of this process would be formed a series of three or more interlocking rings, each at right angles to its successor (Fig. 3 *E*, *F'*).¹ How many such successive rings may be formed is not known. Double rings seem to be the most frequent; but in *Chorthippus* (*Stenobothrus*) both Robertson and Wenrich describe and figure triple rings including at least one case in which the lateral arms are long enough to form a fourth ring, though their ends are in fact free. Janssens's diagram (Fig. 1) represents four complete rings with lateral arms at both ends of the series; and it is quite possible, as McClung has suggested, that some of the forms that have been described as twisted or strepsinema stages may really be early conditions of such multiple rings.

Janssens has found that in the heterotypic division the double or multiple ring-tetrads lie on the spindle with their longer axis transverse to that of the spindle, and establish a lateral (atelomitic or non-terminal) attachment; and since successive rings are always at right angles to one another they lie alternately either in the equatorial plane of the spindle or in a plane at right angles to it, *i. e.*, tangential to the spindle. In the ensuing division the series is cut straight through in the equatorial plane (as shown in Fig. 1), rings which lie in this plane being split lengthwise while those lying tangentially are cut crosswise. This curious result is perfectly in agreement with Robertson's observations on *Chorthippus* ('16, Figs. 179-182) and those of Wenrich on *Trimerotropis* ('17, Plate 3, Figs. 17, 18), and we may probably accept it without hesitation, at least for some tetrads of this type.

Thus far all observers are in agreement concerning the external structure and mode of division of the compound

¹ This account does not correctly describe the mode in which these compound rings actually arise, but it is a convenient way of making clear their structure.

rings. As soon as we look further we encounter what seems at first sight to be a hopeless contradiction between the conclusions of Janssens and those of others.

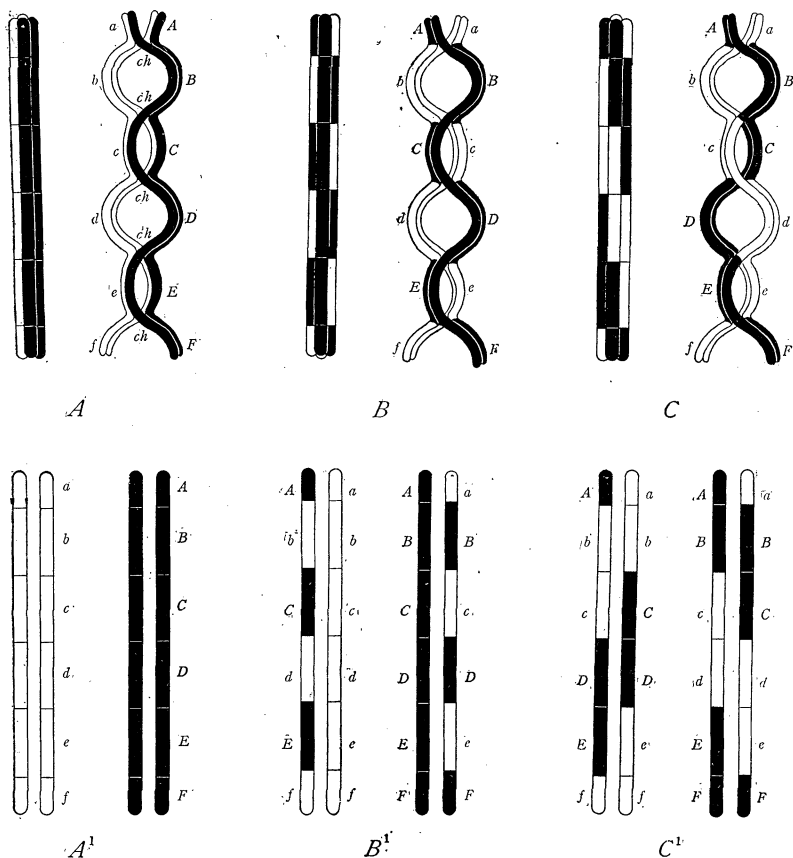


FIG. 4. Diagrams illustrating various possibilities concerning the compound rings, following the outlines of Janssens's figures, but showing also the relations of the chromatids. At the left in each of the upper figures is the longitudinal tetrad-rod from which the ring-series arises, showing results of assumed early cross-overs in B^1 or C^1 . A , the compound ring as conceived by McClung, Robertson, etc., with the four resulting chromatids at A^1 (no cross-overs). B , a compound ring, such as might follow a two-strand cross-over at each node, giving the results shown in B^1 . C , a compound ring giving the results shown in Janssens's diagram (Fig. 1), resulting from a two-strand cross-over between two pairs of threads, in regular alternation at successive nodes. The result (C^1) is four classes of chromatids, as shown in C^1 .

Janssens, holding fast to the general interpretation outlined in his earlier development of the chiasmatype-theory, considers the compound rings to have resulted

from a process of torsion of the synaptic mates about each other, followed by a partial fusion between them at certain points where threads from opposite sides of the spiral have come together, crossing each other to form a "chiasma" at each such point. By a subsequent readjustment of position the regions between these points of partial fusion have opened out to form rings disposed at right angles to one another, and connected at the points where the chiasmata have been formed. The general nature of this rather complicated conception may better be grasped by a study of Fig. 4C than from a description. Janssens assumes, further, that *at some period* in their history the rings are cut through at these points of fusion in such a manner as to effect an exchange of corresponding regions between the synaptic mates. The effect, as conceived by Janssens, is shown in Fig. 1 (copied from Janssens), and more in detail in my interpretative Fig. 4 C, C'.

Janssens's general interpretation (as will at once appear from his diagrams here reproduced as Figs. 1 and 2 A-E) includes two more specific assumptions on which the whole matter turns. These assumptions are: (1) that all the rings are essentially alike, the synaptic mates, or corresponding regions of them (black and white in the figure) lying in every case on opposite sides of the ring-opening, the longitudinal cleft in each thus representing the future equatorial-division; from this it follows (2), that rings which lie in the equatorial plane of the spindle (horizontally) are divided equatorially, while the alternate rings that lie tangential to the spindle are cut crosswise, and hence reductionally, *by the same division*. Both these assumptions differ wholly from the results of previous investigators and hence call for critical examination.

The genesis and later history of the compound (especially the double) rings has been most fully studied in the Orthoptera, having first been considered by McClung and Granata, and more recently investigated with greater

precision especially by Robertson ('16) and by Wenrich ('16, '17). None of these observers, it is true, has traced the history of the rings in every detail; but their results, so far as they go, are entirely in harmony with the better known history of the single rings and double crosses,

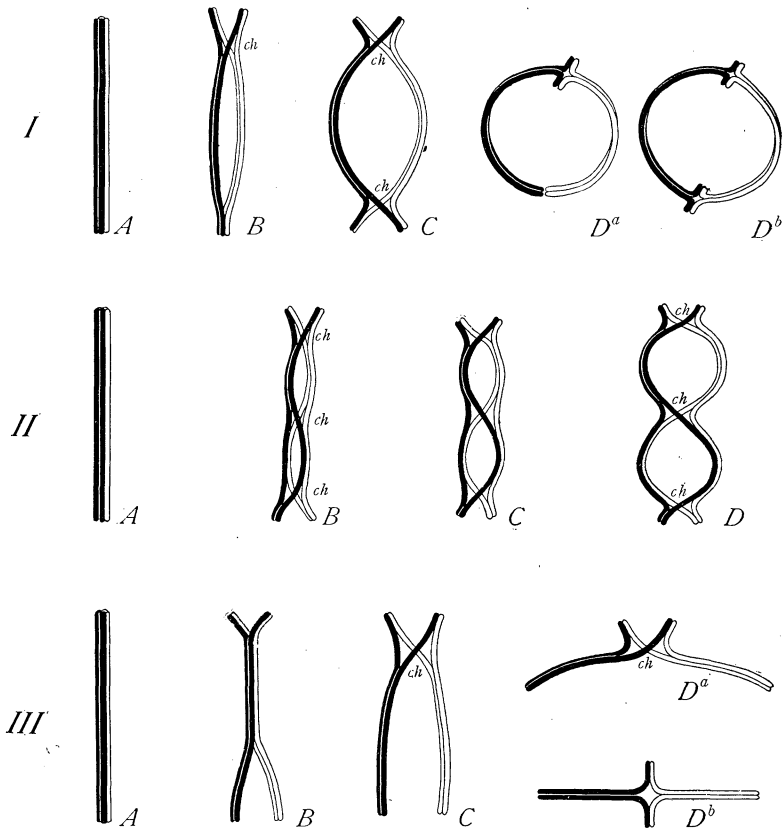


FIG. 5. Diagram (perspective views, from clay models) of the origin of single rings, double rings and double crosses from a longitudinally quadripartite rod. I, single rings; B leading to D^a , and C to D^b . II, double ring-formation. III, double cross-formation; III, C derivable from I, B by separation of the lower ends of the synaptic mates. In each case *ch* marks an apparent crossing-point or "chiasma."

both of which offer essentially the same problem as the compound rings. These various forms of tetrads arise from a diplotene thread that is at first longitudinally double and sooner or later longitudinally quadripartite

owing to the appearance of a second cleft at right angles to the first. The evidence is nearly or quite conclusive that one of these clefts coincides with the original plane of synapsis or side-by-side apposition of the synaptic mates (also the plane of the future reduction-division) while the other is the equation-plane along which each synaptic mate is longitudinally split.² In any case it is generally agreed that single rings arise by the separation and opening out of these threads along *one* of the clefts (generally believed to be the synaptic, as in Fig. 5 I), their ends remaining united, while the second cleft remains as the longitudinal cleft of the ring and represents the plane of the equation-division. The lateral arms of these rings arise, as shown in the figures (5 I, B, C) by separation and divergence of the free ends for a certain distance along the second (equational) cleft, thus finally giving the appearance of a double cross at this part of the ring (5 I, D).

Double rings, coupled together (Fig. 5 II) arise when the rods separate along different planes in two adjoining regions, the opening of one ring representing the expanded synaptic cleft (appositional or reductional) that of the other the equation-cleft. Such rings are of course at right angles to each other; and as the diagram shows (Fig. 5 II, B, D) when these tetrads are viewed obliquely they seem to show at certain points crossed threads or chiasmata (*ch.*) in which two threads cross over from opposite sides.³ It is of the first importance, however, to bear in mind the fact that *such figures are shown in fore-shortened view*. They are an attempt to represent in two dimensions a figure which actually is in three dimensions. Such tetrads can not adequately be visualized until modeled in clay or by means of wires, so as to be seen in three dimensions. When the models are obliquely viewed they seem indeed to show at each node

² This interpretation disregards the possibility (which I think is a probability) that recombination-phenomena or orderly exchanges of material between the synaptic mates may already have occurred in the quadripartite rod; but for the moment we may leave this out of account.

³ This is clearly shown in McClung's photograph, Fig. 122 ('14).

two threads that are connected by a chiasma and two that are not thus connected; but if the model be rotated through an angle of 90° the appearance is reversed, the "chiasma" now appearing between the two threads that previously seemed unconnected, and *vice versa*.

The same appearance, due to the same cause, is given in early stages of the lateral arm-formation in the single rings (5 I, *B*, *C*), and is shown with even greater clearness in early stages of the double crosses. The latter arise by separation of the free ends of the four threads from each end towards the middle point, but along different planes (Fig. 5 III, *B-D*), *i. e.*, from one end along the equation-cleft, from the other along the reduction-cleft—a process that is continued until all four threads come to lie in a single plane in the form of a double cross. Here, too, a "chiasma" (*ch*) is very clearly seen; but as in the foregoing cases it is an optical illusion; the models in three dimensions show at once that a straight split through the tetrad involves no transverse break in the chiasma, and that its two strands merely draw apart as the division proceeds. In themselves these figures give no reason whatever to assume that such a break (crossing-over) has taken place at an earlier period or that the synaptic mates have been twisted about each other, as Janssens assumes.

Such an origin of the double or multiple rings seems at first sight wholly inconsistent with Janssens's interpretation; for if it be correctly determined the relation of the synaptic mates to the ring-formation is wholly different in successive rings, as is shown in Figs. 3 *D*, *E*, 2-*H*, and 4-*A*. Specifically, in case of any two successive rings one always shows the synaptic mates, lying on opposite sides of the ring-opening, and each longitudinally split, while in the adjoining ring half of each synaptic mate surrounds the entire ring-opening, lying in close contact with the corresponding half of its mate. Only in the first case, accordingly, does the longitudinal cleft of the ring correspond to the equation-division. In the sec-

ond case this cleft coincides with the apposition-plane of the synaptic mates (*i. e.*, that of the future reduction-division) while the equation-cleft has opened out to form the ring-opening; and so on in regular alternation. It follows, lastly, that if we disregard for the moment the possibility of an earlier recombination-process, a division that cuts straight through the tetrad, as described alike by Wenrich, Robertson and Janssens, does not in fact divide certain rings equationally and others reductionally in regular alternation but divides the whole series in the same way, either equationally or reductionally as the case may be (Figs. 2 *H, J*, 3 *E, F*, 4 *A*).

In order to make clear the contrast between this conclusion and that of Janssens I have in Fig. 4 *A* followed his outlines but have indicated the course of the four threads (chromatids) in accordance with the account just given. In Fig. 4 *C*, on the other hand, the chromatids are shaded black and white in such a manner as to fit with Janssens's account. A similar comparison is shown for the double-ring tetrads in Figs 2 *F, G*, which follow Janssens's outlines (2 *A, B*) as nearly as possible but are differently shaded; while 2 *H-J* shows the double ring and its mode of division in slightly oblique view, so as to show the "chiasma." In these various figures it is at once evident that although a two-strand chiasma or crossing (*ch*) appears at the junction of every two rings, a straight longitudinal division of such a tetrad (separating black from white) involves on crossing-over, and divides every ring reductionally; *i. e.*, in such a manner as to disjoin the synaptic mates. Here again it is also evident that the multiple ring need involve no twisting of the synaptic mates about one another. It is true that rings of this type, whether single or double, are not infrequently twisted in their earlier stages, and sometimes in their later—a fact long known and easily verifiable; it is shown unmistakably, for instance, in some of my own slides of *Phrynotettix* (from material given me by McClung several years ago). No evidence has yet been pro-

duced, however, to show that such torsion leads to double ring-formation by a process of chiasmatty. On the contrary, the evidence thus far indicates that the torsion is undone as the prophases advance; and it is a significant fact that in these same twisted rings the free ends of the chromatids (forming the lateral arms) show the typical relation as described above, giving the appearance of a chiasma at each end (as in Fig. 3 *E*, *F*, or 5 II).⁴ Such "chiasmata" (like those seen at the junction of two rings) are not for a moment to be confused with the appearance of crossed threads given in side views of actually twisted rings.

Such is the contradiction—at first sight it seems irreconcilable—between Janssens's conclusions and those of other investigators of these tetrads. These latter results, in particular those of Robertson and of Wenrich, are supported by very detailed and precise studies; and my own observations, particularly on the double crosses, are altogether in favor of their conclusions. Until Janssens's evidence is before us in greater detail it remains to be seen whether the contradiction really is as great as it now appears. In the meantime we may briefly consider certain possibilities which may help to define the issue more clearly.

The conflict of results has not, I think, grown out of the fact that Janssens has worked with a different type of compound ring, though this is possible, nor can we assume that he has not reckoned with the results of other observers. I incline to think that the contradiction may be in the main one of theoretical interpretation rather than of known fact; for in theory all the observed facts may quite logically be interpreted as the result of a chiasmatype that has been completed at a stage prior to the ring-formation. Specifically we might assume that a cross-over has earlier been completed at each node in the series, causing an exchange of two longitudinal halves in

⁴ See for instance Granata ('10, Fig. 29), Robertson ('16, Figs. 150*b*, 175), Wenrich ('17, Pl. I, Figs. 8, 9), and Mohr ('16, Fig. 131). The same is clearly seen in my slides.

alternate rings—a process which would produce a condition identical so far as appearances go, in both structure and mode of origin, with the compound ring as described by Granata, McClung, Robertson or Wenrich, but one which has a quite different morphological significance. Such an assumption seems to me to be logically implied by Janssens's own account, though I am not sure that such is actually his meaning.

I have tried to illustrate this by the series of diagrams shown in Fig. 4. *A* represents the compound ring in accordance with the results of McClung and his followers, the synaptic mates being in black and white, respectively. If, however, we assume this condition to have been preceded by a two-strand cross-over or chiasmotypy at each node, the composition of the tetrad becomes that shown in *B* or *C*. Either of these figures realizes the two specific assumptions of Janssens earlier emphasized, namely: (1) that the longitudinal cleft in every ring represents an equation-division (*i. e.*, separates corresponding halves of one synaptic mate in this particular part of the tetrad), and (2) that a straight split through the ring-series (such as is shown in Fig. 1 *B*) will now divide half the rings equationally and the alternate rings reductionally. Both show recombinations in the same regions (*Aa, Cc, Ee*) and in the same relative numbers in the cross-over threads; but they are differently grouped, owing to the fact that in one case (*B*) a cross-over has taken place between the same pair of threads at every node, while in *C* this occurs only at every other node, the cross-overs taking place in regular alternation between two different pairs of threads. As a comparison of Figs. 2 and 4 *C* will show, it is this latter form that corresponds with Janssens's interpretation.

Janssens does not make it clear in his preliminary papers whether he assumes the chiasmata to be cut through during the actual division of the tetrad, though I think is what one would naturally infer from his general account and from his figures, especially of the double

rings (here reproduced as Figs. 2 *A-E*) and of the double crosses (here Figs. 6 *H-C*). On the other hand a study of my Fig. 4 will show that the results of the division, as stated by Janssens himself (my Fig. 4 *C*¹) can only be brought about by a split which passes straight through the equational cleft of the horizontal rings and leaves the chiasma untouched. I infer, therefore, that Janssens does in fact consider the chiasmotype to have taken place

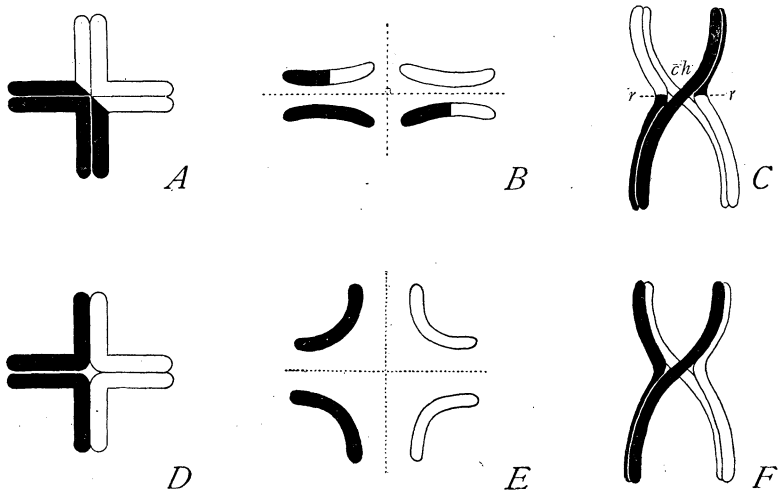


FIG. 6. Interpretations of the double crosses. *A, B*, Janssens's interpretation (from his figures), showing four classes of chromatids, two with a single cross-over each. The result is here the same as shown in Fig. *C* (after an earlier figure of Janssens). *D, E*, the prevailing interpretation of the double cross, with no cross-overs; *F*, early stage of the double cross. (Cf. Fig. 5 III, *C*.)

at a stage prior to the opening out and division of the rings; and this would be in agreement with his earlier conclusions, as applied to the tetrads of urodeles, here illustrated by Fig. 6 *C* (after Janssens).⁵ At any rate, so far as I can see, it is only by such an interpretation that Janssen's results can be reconciled with those of other observers. More specifically, the assumption must be, I

⁵ See Janssens, '09, p. 14 (Diagram, XXII). "We believe that in this case the threads which cross each other are those furthest apart, that is to say, which occupy those parts of the chromosomes that undergo no intermixture. The threads which remain unconnected by a chiasma, on the contrary, are those which have undergone a secondary union at the points where the chromosomes have interpenetrated each other and fused."

think, that the chiasmotypy has taken place during a strepsinema stage prior to the straight, longitudinally divided threads from which the rings arise (Figs. 4 *B, C*, at the left). If now, for the sake of argument, we accept these assumptions, how does it come to pass that the subsequent opening out of the rings exactly fits with the recombination-phenomena that have previously occurred in the tetrad? Morgan has already supplied an answer to this in the ingenious suggestion that *the mode of separation of the threads may be determined by their nature* —i. e., that the paternal and maternal threads (or portions of threads) may always be the first to separate, however they may lie in the tetrad.⁶ This is an important addition which makes the whole series of assumptions logically complete.

All this constitutes a somewhat complicated train of reasoning; nevertheless, if it be granted, it provides formally an escape from the seeming contradiction and leaves the chiasmotype-theory intact. The point, however, that I wish to emphasize is that we have now passed over into a realm of hypothesis and logical construction, based it is true on a vast assemblage of data of the highest importance, but derived from genetic experiment rather than from cytological observation. No observer, so far as I know, has yet seen a process of true crossing-over (recombination) by means of torsion, chiasma-formation, fusion, and secondary splitting apart. That such a process takes place at all remains thus far an inference based on the presence of a continuous two-strand chiasma in later stages of meiosis and on certain resulting appearances in the late prophase- and metaphase-tetrads. But as shown above, precisely the same appearance of a two-strand chiasma is given by a process in which no torsion need be involved. Both Wenrich and Robertson have urged this fact against Janssens's interpretation; and I am fully in agreement with them so far as the later stages of meiosis are concerned. It may nevertheless be pointed out that both these observers have figured stages

⁶ '19, pp. 101-104.

which at least suggest a process of torsion or strepsinema-formation in the early diplotene prior to, or very early during, the definitive opening out of the prophase-figures—*e. g.*, in Wenrich ('16), Fig. 75, or ('17), Fig. 23, and in Robertson ('16), Figs. 149, *a* and *b*. The case seems, therefore, by no means closed; and we may await the publication of Janssens's new results in greater detail, in the hope that more definite evidence may now be produced concerning the critical point at issue.

My own doubts on this matter first grew out of observations on the origin of the double crosses, which, as above indicated, involve a similar question concerning the chiasmatype. Janssens's earlier interpretation of the double cross, which I believe he was the first to offer, was in principle the same as that briefly indicated above and schematically shown in Figs. 5, III and 6 *D-E*. Later this interpretation became the prevalent one but was abandoned by Janssens himself ('09, '19) in favor of one which assumes a process of chiasmatype to be involved in the cross-formation. This interpretation starts with a comparison of the double cross to the region at which two rings join; and this is obviously correct under any theory (cf. Figs. 3 *D* and 6 *C, F*). Janssens, however, assumes the relation between the synaptic mates to be essentially as shown in the diagram here reproduced as Fig. 6 *A-B*, the two synaptic mates being bent at right angles, and united by their apices to form a cross which then splits straight through all four arms, thus giving two cross-over chromatids out of four. I seriously considered this interpretation in my own studies on the double crosses of Hemiptera, but finally became convinced ('12 and subsequently) that it does not correspond with the facts. More recently Robertson, Wenrich and Mohr have demonstrated the same conclusion in a very circumstantial and convincing manner in case of the double crosses of Orthoptera, tracing their origin step by step from the original diplotene in the manner indicated in Fig. 5, III. According to all these observa-

tions there is nothing in the history of these crosses, as thus far made known, to suggest an earlier process of torsion, chiasma-formation, and recombination. They indicate rather that the cleavage of such a tetrad straight through its two clefts involves simply one reduction-division and one equation-division (Fig. 6 *D-E*).

Robertson has pointed out that many of those appearances in the prophase- and metaphase-tetrads on which Janssens's theory was originally based are susceptible of a much simpler explanation than is offered by the chiasmatype-theory, namely, that they are a result of "misfortune in the prophase," due to secondary displacements of torsions at this time. Experiments with clay models have convinced me that this point is well taken, in respect to some at least of these appearances. It should also be clear from the foregoing discussion that conditions resulting from the persistence of the so-called two-strand chiasma in the metaphase-figures are readily explicable without the assumption of an earlier process of chiasmotypy.

In this brief review and critique of the cytological aspects of the question, I have not intended to take up an attitude of opposition towards the chiasmatype-theory considered as an explanatory principle in genetics. On the contrary, I am not able to escape the conviction that somewhere in the course of meiosis some such process must take place as is postulated by Janssens and by Morgan and his co-workers, though I must admit that this opinion rests less on cytological evidence than on genetic. I have wished only to discuss the possibilities of the existing cytological situation and to offer a counsel of caution in respect to the chiasmatype-theory in so far as it is based on conditions seen in the later stages of meiosis. This means no lack of appreciation for Janssens's brilliant and fruitful work, which has opened up so remarkable a new field of inquiry. But a theory of such fundamental importance calls for critical treatment, and on its

purely cytological side too much has sometimes been taken for granted by writers on genetics. It is, I think, highly probable that the cytological mechanism of crossing-over must be sought in some process of torsion and recombination in the earlier stages of meiosis—perhaps during the synaptic phase of slightly later—and that this process may leave no visible trace in the resulting spireme-threads. To accept this, of course, would mean that such conventionalized diagrams as those here offered (Figs. 3, 5, etc.) should be so modified as to indicate exchanges which have earlier taken place between the synaptic mates. It must be said, on the other hand, that the actual evidence of torsion during the process of parasynapsis is still very inadequate and receives no support from some of the most careful recent work. One can not avoid a suspicion that some internal process of torsion (or of rotation, as conjectured by Correns) may take place in the early pachytene before the duality of the diplotene becomes externally visible. Conjecture concerning all this will however be less fruitful than further cytological analysis. The truth is that for the time being genetic development of the chromosome-theory has far outrun the cytological. We are in no position to predict when the plodding progress of cytology may be able to close the gap: nevertheless we have every reason to hope that the physical mechanism of the recombination-phenomena may in the end prove to be accessible to decisive cytological demonstration.

THE SPIRAL LOOPING OF THE CHROMOSOMES AND THE THEORY OF CROSSING OVER

T. H. MORGAN

In his two recent papers Janssens calls attention to certain details relating to the application of the findings of cytology to the interchanges between homologous sets of linked genes. The first paper is a restatement of the situation as it is generally understood to-day, and calls

for no special comment. It ends with the significant statement

At our next meeting I shall point out that the theory of the chiasma-type allows for an interpretation somewhat different from the view of simple splitting of the threads in a single plane that passes through the axis of the entwined threads.

Concerning the point here raised by Janssens I should like to add that the "simple interpretation" was given mainly to escape the somewhat complicated scheme involved in Janssens's theory. In this way it was hoped to avoid a too detailed account of the process that calls for pictures not readily understood except by cytologists familiar with the changes that take place when the twisted threads shorten and move apart. Unfortunately the very simplicity of the statement led one critic to infer that the interpretation must be wrong because at the nodal points the plane of the split appeared as though it cut obliquely through each chromosome itself. To avoid this I represented in later diagrams the chromosomes as made up of beads, and in this way tried to show that at the node each bead and its allelomorph are not divided, but go each to a pole. Even this diagram may prove too simple; for, if at the time of twisting each thread is also split lengthwise into two strands it is possible that only two of the strands fuse at each node. In Janssens's scheme to be described below this secondary doubling of each thread is seen to be an important factor in the situation.

Janssens states that the matter is not simple:

The loops (Fig. 1 *B*) and the half loops (Fig. 1 *C*) that produce the chiasma lead to profound modifications in the twisted threads. These modifications are already indicated in the prophases, but they only become evident in proportion as the dyads ripen and prepare to place themselves on the spindle. We can not describe this here, but let us state nevertheless that the chiasma segments are placed in planes perpendicular to the segments adjacent to them as indicated in diagram II, Fig. 1. Once this fact is clearly seen it is not essential to add much to Morgan's phrase, since it expresses what really takes place. It need only be said: (1) In both maturation divisions a cleavage takes place

only in the equatorial plane of the figure. The first of these cleavages is here indicated by a dotted line in Fig. 1 *A*. (2) Moreover this plane produces a longitudinal cleavage of the chromosome and hence is equatorial in each of the alternating chromosome segments (rings) that lie exactly at the equator of the spindle of the figure (like that which occurs at a gonial mitosis), Fig. 1 *A* and *B*. (3) Finally, since the two spindles of the two maturation divisions that follow rapidly are perpendicular to each other, one may further add that each dyad will be split during the maturation (maiotiques) division by two planes at right angles to each other. At the first division, the equatorial division plane is perpendicular to the axis of the heterotypic spindle and during the second division the plane is in space, parallel to the original axis of the same spindle.

Janssens suggests the following considerations that have an application to Mendel's laws.

Neighboring segments pass easily into the same chromosomes when the direction of the twist is constant. When a segment is long it may be considered as carrying a longitudinal series of qualities, in conformity with the ideas held by Morgan. On the other hand the qualities supposed to be carried by the chromosomal segments are distributed amongst the germ-cells as though they were carried by chromosomes really independent confirming the law of disjunction of the characters in the gametes (Mendel).⁷

Let us return to a further consideration of the diagrams that have been published to represent the methods of crossing-over. In Janssens's scheme, Fig. 1 *A*, four complete rings are represented and the division plane appears to cut through each node, although the important details of how this is done are not shown in the figure, but may perhaps be inferred from Fig. 1 *C*, where the four vertical strands show what is supposed to have taken place. Crossing-over is represented as having occurred at four nodes. In *Drosophila* the genetic evi-

⁷ In this sentence Janssens seems to imply that his chiasma theory explains "free assortment" between genes in homologous pairs of chromosomes, but obviously if the genes are in linear order, great numbers of them will go over together in the segments between the nodes, or on each side of a node. Hence the phenomena of linkage that places a very great restriction on Mendel's second law of assortment. It is this feature that we have always regarded as of the utmost significance in our theory of crossing over. It is obviously implied in Janssen's chiasma theory also, and I can not but believe that Janssens must intend to apply his theory in the same way in which we have applied our theory.

dence shows that as much crossing over as this does not usually occur. In our diagrams (Heredity and Sex, 1913), therefore, we represent only one or two real interchanges between the members of a pair of chromosomes because the genetic evidence shows, as stated, that, in the great majority of cases, this is what takes place.

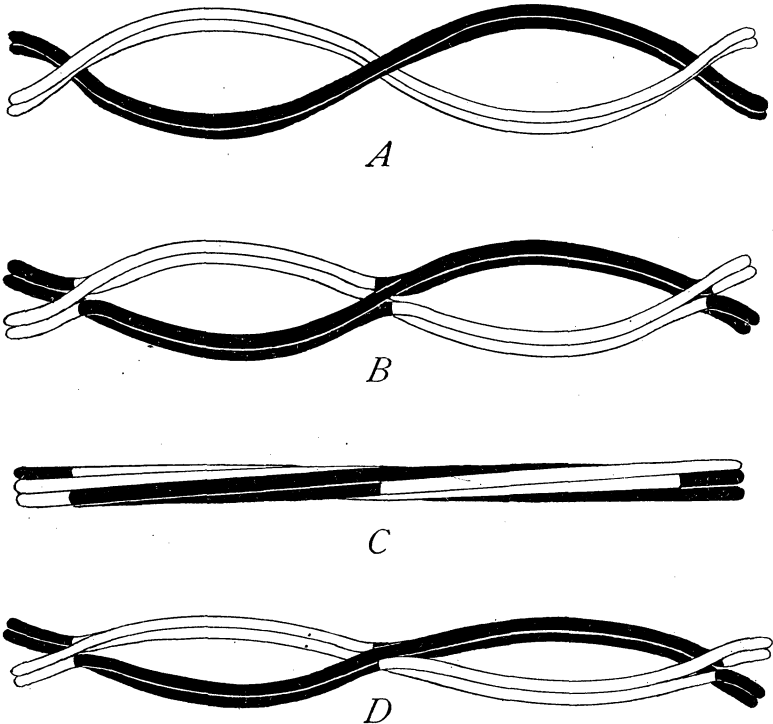


FIG. 7. Diagram of the looping of a pair of chromosomes that are already split lengthwise. *A*, two threads making one complete twist; *B*, the inner strands of each thread interchange at the crossing points; *C*, the threads next flatten against each other, still keeping their twisted configuration; *D*, just before, or after the conjugated thread enters the spindle it opens out by a reduction split that takes place in each segment of the thread. If the strands still keep their spiral relation, two strands cross over at each node; at the ends it is the non cross-over strands that cross, in the figure, but it is the other strands in each case that have broken and "crossed over." The crossed strands in the figure are not due to perspective.

The rings in two planes, as represented in Janssens's diagram, call for further analysis. We may call these rings *Bb*, *Cc*, *Dd*, *Ee* (Fig. 1 *A*). It will be observed that

in ring *Bb* the dark double thread (half ring) at the right separates from the light double half at the left. This is a reduction division for this segment. On the other hand in the ring *Cc* the division plane separates equationally the halves of the dark and of the light half rings. Crossing over takes place at the node between ring *Bb* and *Cc*, and at the node between the rings *Cc* and *Dd* there is another crossing over between the other two strands. Generalizing the result it may be said that crossing over of two of the strands takes place at each node. In the second division, that is supposed to take place here in the plane of the paper, there is assumed to be no further crossing over in either of the halves that have resulted from the first division.

Janssens points out that on this new scheme there is only half as much crossing over as on the scheme represented in our older diagram (1913); but it is obvious that this is only because in the latter whole chromosomes (each potentially or actually made up of two strands) are represented as crossing over at each node. If, however, we compare this latest scheme of Janssens with the figures that we have now recently published ("Physical Basis of Heredity," 1919) in which, following some of Janssens's earlier diagrams only two of the strands cross over at each node, it is perfectly clear that these later schemes of ours give the same number of cross-overs per complete twist as do Janssens's present diagrams.

It may, therefore, not be without interest to compare Janssens's latest scheme with the one I have recently suggested in my book on the "Physical Basis of Heredity" (p. 105) where a figure is given that suggests an explanation of the opening out of a twisted conjugated thread in rings that lie in different planes. This figure is here reproduced, Fig. 8 *A-D*, modified only so far as to make it comparable with Janssens's new diagram. In *A* the two split threads are represented as looping or overlapping in an open spiral (an earlier stage than

Janssens's first figure). At this stage where the inner strands come into contact they are represented as fusing with each other at three nodes. The threads may next be supposed to flatten against each other to make the conjugated threads keep their spiral configuration and then condense to make the thick threads. In this condition they pass to the equator of the spindle or they may begin to open out before they reach the equator, Fig. 7 *B*.

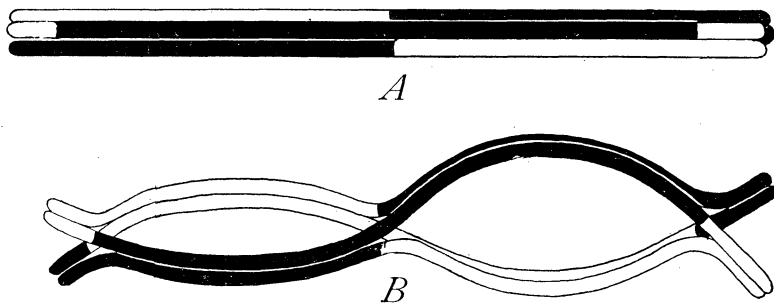


FIG. 8. Diagram showing how the twisted strands of Fig. 8, *C*, become straightened out (untwisted) as the thread shortens, so that the former spiral relation is lost. The resulting relation of the threads when they open out by the reductional separation is shown in *B*. *A*, this figure shows the relation of the strands when the spiral in Fig. 8, *C*, untwists, *i.e.*, as the thread shortens; *B*, if the thread shown in *A* now opens out by a reductional separation in each segment, the resulting figure, *B*, is the same as that of Janssens. In the middle of the figure it appears as though two "cross over" strands were crossing. This is here due to perspective.

If the first division is reductional for every part of the thread, the halves of the thread move apart in opposite directions, and as a consequence of the way the twisted threads have flattened against each other this opening out may produce rings lying in different planes, Fig. 7 *D*, not necessarily at right angles to each other, but at an angle with each other.

The rings are assumed to be due to the reductional separation of the segments of the chromosomes along the tetrad, but the further movements of the daughter chromosomes after they have reached the equator of the spindle must be referred to another mechanism that now comes into play, namely, the forces that carry the chromosomes to the poles. Under these circumstances the

threads may be thought of as separating without assuming such a strictly symmetrical form as Janssens's new diagram indicates, or in other words the separation of the chromosomes may take place as Janssens described it in *Batracoseps*. The suggestion that I made to account for the appearance of rings in different planes was made to meet an objection raised by Robertson and by Wenrich, namely, that the crossed threads (the chiasma threads) do not mean that crossing over has taken place in that region. They point out that the crossed threads may mean no more than that a *not-twisted* tetrad has opened out in different planes in consecutive regions. This obviously may be the interpretation of the crossed threads, but if as I suggested the opening out of the rings themselves in different planes represents consistently a reductional separation in a formerly *twisted thread*, then the cross threads come to have a meaning, for they represent the level at which an earlier fusion and reunion of the inner strands of the four strand stage took place. From this point of view the cross strands, while having nothing to do *at this time* with crossing over, nevertheless correspond to levels at which that process occurred.

I do not wish to appear to be advocating the scheme that I suggested as the best or as the only one that is involved in crossing over. Any scheme that accounts by means of twisting threads for interchange between the segments of homologous chromosomes will fulfill sufficiently the present requirements of crossing over. Much more cytological and genetic work too will be necessary before it is possible to state when and how this process goes on. One point alone seems at present to be indicated with some probability by the genetic evidence, namely, that it would appear simpler for the interchange to take place when the lines of genes are extended to the fullest extent possible, and this would seem most easily to take place, in the accurate way indicated by the genetic facts, when the leptotene threads have spun out to their farthest extent. Whether Janssens also ascribes to this

stage the essential step in the breaking and reunion of the strands remains to be seen when his new results are published.

Until Janssens publishes a full statement as to how he supposes the crossing over at the nodes to take place, whether at the time when the looping of the threads is present, or at an earlier stage, it is hazardous to make too detailed comparisons, but one relation should not pass unnoticed. In Janssens's figure four rings seem to be involved in one complete twist of the two chromosomes. In order to place these rings in such a position that a single (vertical) plane can sunder successive rings transversely and longitudinally in alternation, the rings must be turned so that two are exactly vertical and two are horizontal. A spiral relation of the threads can not be brought into this relation unless the threads first untwist. How this can be done is shown by a comparison of Fig. 7 with Fig. 8. In Fig 7 *A*, as explained, two chromosomes, each of two strands, are represented as looped around each other in an open spiral. In the middle of the spiral the two inner strands that touch are represented as fusing and reuniting to give the cross-over, and near the ends, where the threads cross again, the other two strands fuse, break, and reunite to cross over, Fig. 7 *B*. The threads are then represented as flattening against each other, still keeping their spiral configuration. When they open out again, by a reductional separation of the segments, Fig. 7 *D*, the rings are formed, and if the threads are still represented as keeping their spiral configuration no single plane, as explained, will separate them without cutting some of the strands. But if when stage *C* is reached in Fig. 7 the threads straighten out as they condense (*i. e.*, if they untwist) the result will be that shown in Fig. 8 *A*. If now the threads open out by a reduction division in each segment, the resulting figure will be like that shown in Fig. 8 *B*. This figure is the same as that of Janssens, and the halves can be separated in one plane, as he explains. We may conclude then, if the con-

jugated threads after crossing over do not untwist, they will give figures like those in Fig. 7 *D*, and such threads must be pulled apart as Janssens has explained for *Batrachoseps*; but if after crossing over the twist is rectified as in Fig. 8 *A* the threads can separate as Janssens explains for the grasshoppers. In both cases the crossing over is represented as the result of twisting threads, and if such loops tend to have a modal length, the mechanism furnishes a beautiful explanation of interference which is one of the crucial tests to which our explanation of crossing over has been put.

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