THE STRUCTURE AND BEHAVIOUR

of

CHROMOSOMES DURING MEIOSIS

in

TRILLIUM ERECTUM L.

by

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INTRODUCTION.

Genetics and Cytology have, in the past twenty years, combined to place the chromosome in the important rôle of chief physical link between parent and offspring. Through this body the parent bequeaths its potentialities in the form of determiners which have been called genes. Any portion of an organism which has a function of such significance is obviously a subject for considerable investigation. The study of chromosomes is definitely a part of the somewhat wider study of cell processes. The phenomena of mitosis and cell division certainly involve changes in cell physiology and a knowledge of these changes may well be expected to throw some light on the more intimate nature of protoplasm. The study of chromosome structure, therefore, falls logically into its place as one of the many ramifications of the biologists' main problem, the establishment of the laws governing living matter. The problem of chromosome morphology is, therefore, of general biological significance. In addition, recent developments in Genetics tend to give it a more specific significance. The discovery, or perhaps more accurately, the realization that the arrangement of the genes is of genetical importance suggests that a consideration of the chromosome as a whole is not without a potential genetical value. While no attempt has been made towards seeking a genetical significance in the structures studied during the present investigation, yet it is,

perhaps, well to keep in mind that there may be such significance. In the past few years it has become increasingly clear that we can not afford to overlook possible connections between the structural peculiarities of chromosomes and the genetical behaviour of organisms.

The complexity of the chromosomes was first indicated by Barenetzky's (1880) discovery of a coiled filament in the chromosomes of living pollen mother cells of Tradescantia. Unfortunately the importance of these observations was not immediately realized and over forty years elapsed before any real effort was made towards solving the many problems of chromosome structure and behaviour.

In 1926 the almost simultaneous publication of several important papers on morphology by Kaufman, Kuwada and others together with the publication of Belling's aceto-carmine smear technique initiated a period of intense work along morphological lines. In the twelve years which have since elapsed great gaps have been filled in our knowledge and many new problems have been raised. The bulk of these new problems are concerned with details of structure and behaviour rather than with more general matters. On such problems as the gross behaviour of chromosomes through division there is very good agreement among cytologists but on questions of structural detail many conflicting opinions have been expressed. Such problems as the origin of chiasmata, the major spiral and

relational coiling; the presence of the minor spiral; and the number of threads at various stages still remain controversial matters.

In 1935 Huskins and Smith published a paper on the structure of meiotic chromosomes in Trillium erectum L. Not the least important part of this contribution was the fact that the advantages of this plant as a source of critical cytological material were established. The chromosomes are large, varying from 8-15μ in length at first metaphase, and are easily distinguishable from one another at metaphase and anaphase. The four strands at diakinesis and metaphase may be seen clearly and traced through chiasmata and in some cases even through the attachment.

In addition to this published work, Dr. A.W.S. Hunter has added some very valuable information in his analysis of the direction of coiling in synaptic and asynaptic materials. Certain unpublished figures and data obtained by Mr. H.B. Newcombe have also proved valuable in the present analysis.

The aim of this study is to consider the Trillium data accumulated by various workers in this laboratory since 1932 and from its analysis to attempt the derivation of some of the factors underlying various aspects of chromosome structure. The chief problem for consideration at present is coiling, both spiral and relational, since it is this problem which has received the most attention in this laboratory up

to date. In studying this problem, however, it has been found advisable and often imperative to consider other points which are more or less closely connected with the main study. Such factors as the mechanical attributes of the matrix and the number of threads at various stages are apparently, as will be shown, quite intimately bound up with the question of spiralization.

Certain facts have emerged from this study which have hitherto been unknown and which are, indeed, contrary to established opinion. It is necessary, therefore, to consider the problems to which these facts pertain from a new angle. As a result of this necessity several new hypotheses have been proposed. If at times it seems that too much importance has been attached to these, it is because the data cannot be discussed with any degree of coherence without some connective background. No new hypotheses have been formulated, however, unless, in my opinion, it has been virtually impossible to fit the data to established theory and the hypotheses have been used only in the way which I believe they should be used; that is, as a tentative bond between the various facts associated with a single phenomenon.

REVIEW OF LITERATURE.

Introduction.

A complete review of the literature of the last twelve years on chromosome structure would in itself comprise a sizable volume. It has therefore been deemed advisable to reduce this section to a brief consideration of the most outstanding contributions. Many authors have traced the historical development of the problem from Barenetzky's spiral observations to the present day complexities. Between 1880 and 1926 little was accomplished except the training of workers and the development of techniques. The most significant advance in the latter field was the development of the smear techniques to replace sectioning. A number of workers seem to deserve acknowledgment in this connection but we undoubtedly owe most to Belling (1928) for his useful and rapid iron-aceto-carmine technique. In 1926 Kaufman showed quite conclusively that the continuous spireme of the earlier workers was mythical at least for Tradescantia, since he was able to demonstrate that the chromosome retained its identity through all stages including the resting stage. This discovery served to close an obvious gap in the division cycle. Whether or not this was one of the factors responsible for the renewed interest in the subject or whether outstanding workers were now prepared for a more concerted attack we

cannot say. Nevertheless it was an important step.

In order to avoid as many complexities as possible this part will be divided into two sections. In the first a general outline will be given of the behaviour and structure of chromosomes in mitosis noting as we proceed the more serious differences of opinion. In the second section various opinions on the more specific points with which this paper is primarily concerned will be considered.

Mitosis and Meiosis.

Mitosis.

Since the mitotic process comprises a complete cycle a description of mitosis may be commenced at any stage. There are, however, some advantages in beginning at the stage which is the clearest and concerning which the most is known; that is, metaphase. The description will, therefore, begin at this stage and the chromosomes followed through an entire division.

A. Metaphase. The chromosomes are at least double and the halves may still show some relics of the relational coiling. They have taken up an equatorial position on the spindle and are ready to separate. According to Darlington (1937), following Belar, the distal ends of the chromosomes come into close contact at this time

while the centromere regions separate. Concerning external morphology the chromosomes appear as cylinders of more or less even diameter. The attachment region is marked by a constriction at which point according to Trankowsky (1930) a small body, the centromere of Darlington, may be seen under suitable conditions. Other constrictions may characteristically be present in some chromosomes. On the internal structure of the chromosomes at this stage there is more disagreement due largely to the difficulty encountered in staining for structure. It is, however, rapidly becoming clear that each half-chromosome contains a spiral chromonema. This has been indicated by several workers, and most clearly by Geitler (1935, 1936). As to the number of threads in each half chromosome at this stage there is considerable disagreement. Darlington (1935, 1937) basing his opinion chiefly on the x-ray results of Mather and Stone (1933) and Mather (1934) believes that there is but one thread which remains single until resting stage when it splits. Geitler could find no sign of duality but believes that the double state is probable. Huskins and Hunter (1935) find the metaphase chromosome to be quadripartite in pollen grain division. Hunter (1935) found some chromosomes in root-tips of Anthoxanthum odoratum to be quadripartite at metaphase. Nebel and Ruttle (1936) consider the metaphase chromosomes of Tradescantia and Trillium to be eight-partite; i.e., there are four threads in each half-chromosome. It is possible of course that these different views may not be as widely

divergent as they seem. If, for instance, the chromonema were made up of a vast number of constituent fibres variability in techniques and organisms may well account for the different opinions.

B. Anaphase. Morphologically the chromosomes are similar to those at late metaphase. Opinions differ in the same way as to whether the chromonema is spiralled as maintained by Geitler (1935), Sax (1936), and Darlington (1937), or whether there are two intertwined threads as suggested by Koshy (1933), Warmke (1937), Jeffrey (1937) and others.

C. Telophase. The chromosomes begin to lose their chromaticity and the component chromonemata therefore become more apparent. There is, however, still some argument as to the number of threads and their exact relationship. Koshy (1933) believes that the coils expand but remain intertwined, Nebel (1933) that there are four closely associated coils in Tradescantia, Sax and Sax (1935) that unravelling occurs at this stage while Darlington (1937) considers the chromonemata to be a single spirally coiled thread which retains the coils throughout resting stage.

D. Resting Stage. The chromosomes, according to Darlington (1937), are "not merely permanent but immobile" during this period. There is

much to be said for this view and in many cases it is quite obviously true. Darlington, quoting Taylor (1931) and de Winiwarter (1931), points out that the spiral arrangement seen at prophase corresponds with that seen at the previous telophase. This, of course, strongly indicates structural immobility during resting stage. That the chromosomes do not change their spatial relationships with one another is shown by Belar's (1929b) observations of mitosis in living staminal hairs of Tradescantia. He noted that the polarised arrangement of the chromosomes was the same at prophase as at the previous telophase.

E. Prophase. During this period of the mitotic cycle it is generally agreed that uncoiling of the old coils occurs and that the chromosome contracts in length. It is also obvious that the chromosomes are at least double during this phase (Sax and Sax, 1935; Darlington, 1937; Naithani, 1937). Nebel and Ruttle (1937) find the prophase thread to be four-partite during this stage in Trillium and Tradescantia. Huskins and Smith (1935) also report a four-partite structure in pollen grain division prophase. The chromomeres, which are best seen at leptotene, may also frequently be observed in mitotic prophase. These have variously been interpreted as representing the locus of the gene (Belling 1929), as characteristic artefacts (Darlington, 1937) and as twists in the chromonema (Kaufman, 1926). The more wide spread opinion is one which agrees

essentially with Belling and Darlington. Among other evidence supporting this opinion are McClintock's (1931), photographs of meiotic prophase in Zea which clearly show the chromomeres and Huskins and Smith's (1935) observations of quite distinct differences in sizes of the chromomeres and their unequal distribution along the chromonema in prophase of meiosis. It may also be pointed out that chromomeres of identical appearance may be found at equivalent positions on associated chromatids. Some authors, such as Nebel (1933), consider it possible to confuse true chromomeres with "chromatic specks" which are optical sections of a spiral. He apparently considers both to be present in Tradescantia since he describes the earliest prophase as consisting of four strands which show tightly twisted gyres of small size which eventually expand into a larger spiral at metaphase. Naithani (1937) rules out chromomeres altogether and considers them to represent the gyres of a very fine spiral which develops in much the same way as suggested by Nebel. Sax and Sax (1935) while saying nothing concerning chromomeres, apparently also interpret the chromatic dots as the turns in a spiral.

At late prophase another characteristic phenomenon may be noted; i.e., the relational coiling of the half chromatids. While there is almost perfect unanimity concerning the presence of this type of coiling the questions of its origin, connection with other structural phases and its direction are quite unsettled. A further discussion of this point will be undertaken in the next section.

Meiosis.

The structure of chromosomes during the meiotic division has, largely because of the greater ease with which good preparations may be obtained, received somewhat more detailed study. In spite of this, however, the unsolved problems of meiosis coincide quite well with those of mitosis. For example, there are still differences of opinion concerning the number of threads at various stages and on the origin of coiling. The additional complexities of pairing and chiasma formation also contribute in no small measure to the mechanical problems of chromosome structure.

In the following outline of meiosis, various opinions will be cited as in the description of mitosis.

A. Leptotene. The chromosomes are generally described as long, contorted, optically single threads which show a somewhat beaded structure. While this is the general view, many workers have taken exception to one or more of the details. Because of its theoretical implications the question of singleness of the leptotene thread is the most outstanding point of disagreement. While most workers admit the apparent singleness of the leptotene chromosome there seems to be a feeling on the part of some that this question should be answered by a study of the last premeiotic mitosis. Such a study, however, offers certain

technical barriers since it is difficult in all cases and impossible in some to determine whether any given premeiotic mitosis is the last one or not. Atwood (1937) on Gaillardia and Warmke (1937) on Trillium believe they have overcome the difficulties in these plants. They report that the last premeiotic mitosis differs in no way from any other and maintain that the last premeiotic telophase chromosome is double. That means that the leptotene thread has been split and is therefore structurally double whether or not its doubleness can be optically resolved.

A few workers have reported a double leptotene thread entirely apart from the question of doubleness in the last premeiotic telophase. Among these may be listed McClung (1927), Robertson (1931) and F.H. Smith (1934). Kaufman (1926) and Koshy (1934) have also reported double leptotene chromosomes in Tradescantia and Allium respectively but both these authors have considered the chromomeric appearance to be the result of the looping of two intertwined threads and their interpretation of doubleness must necessarily hinge to some extent on the interpretation of chromomeres. Huskins and Smith (1934) find the leptotene chromosome of Fritillaria Meleagris to be partially double but (1935) those of Trillium to be single. R.M. Love (unpublished) has observed widely split threads in wheat at a stage which he considers to be leptotene. There is some difficulty in proving that this stage is leptotene since it is impossible to count the number

of free ends but it is probably significant that no stage at which the chromosomes were single could be found. Stebbins (1935) considers the leptotene thread to be not only double but quadripartite in Paeonia, Allium and Tulipa and Nebel (1935a, 1936), and Nebel and Ruttle (1936b) make the same observations in Trillium, Tradescantia, Hordeum, Secale and Crocus. The theoretical importance of this point lies in the fact that singleness of the leptotene chromosome at the time of synapsis is the basic assumption for Darlington's (1935, 1937) precocity theory of meiosis as well as for Belling's (1931) theory of crossing over.

B. Zygotene. Synapsis of the leptotene threads begins at this stage apparently at any point along the chromosome, although Darlington (1935, 1937) and Huskins and Smith (1935) report a tendency towards initial association at the attachment and ends. These authors have also observed that chromomeres of corresponding size become associated for the most part although a few exceptions were noted by Huskins and Smith.

C. Pachytene. The chromosomes become obviously four-partite (Huskins and Smith) and crossing threads which apparently are chiasmata may be seen in favourable material. According to Darlington (1937) the paired threads coil around one another and the chromomeres increase in size. Most workers have assumed that the chromosomes contract during these stages but Belling (1931) by measuring

distances between chromomeres finds that they are probably elongating slightly up to early pachytene after which contraction occurs.

D. Diplotene. Opening out between pairs of paired chromatids occurs, complete separation being prevented by the presence of chiasmata (Darlington, 1937). According to Darlington, spiralization generally begins at this period although Huskins and Smith (1935) consider it to occur somewhat later in Trillium.

E. Diakinesis. Coiling and chromosome contraction continue and both reach a maximum during this stage (Huskins and Smith, 1935, and Darlington 1937). According to some authors Kuwada (1934, 1935), Nebel and Ruttle (1937) and Darlington (1937) a small gyred spiral may be seen running at right angles to the major coil. Huskins and Smith (1935) and Warmke (1937) were unable to see this in Trillium.

F. First Metaphase. Except for their position on the equatorial plate the chromosomes at this stage are essentially the same as at late diakinesis.

G. First Anaphase. Separation of half bivalents from each other occurs. The chromatids of the separating dyads remain very closely associated at the attachment but are widely separated elsewhere. The coils may at this time be slightly more compact than earlier (Huskins and

Smith, 1935) but otherwise there is no marked change in structure. Several authors have reported a further split which becomes clear at this time (Huskins and Smith, 1935; Warmke, 1937) while Nebel and Ruttle (1937) consider the anaphase chromosome to be quadripartite. The minor spiral reported by Kuwada and his co-workers in various papers, Darlington (1937) and Nebel and Ruttle (1937) has not been observed by Huskins and Smith or Warmke in Trillium.

H. First Telophase and Interkinesis. The chromosomes congregate at the poles and begin to form new metabolic nuclei. The extent of this transformation varies with different organisms from complete lack of, to complete formation of, an interkinetic nucleus. The appearance of the chromosomes at the second division is modified by the extent to which an interphasic nucleus is formed.

I. Second Division. According to Darlington (1937) when this division follows an interkinesis it is essentially the same as any somatic mitosis excepting that the chromatids are associated only at the attachment. Uncoiling of the major coil takes place and this is replaced either by a new coil (Naithani on Hyacinthus, 1937) or by the minor coil of the first division (Sax and Humphrey on Tradescantia, 1934). If no interkinesis intervenes the second division chromosomes do not differ markedly from those at first anaphase. No further splitting takes place during

this division (Nebel, 1932; Huskins and Smith, 1935; Nebel and Ruttle, 1937).

Spiralization.

The present investigation has necessarily involved some study of all stages of meiosis from leptotene to first pollen-grain division. Nevertheless, the primary problem concerns spiral and relational coils and the phenomena directly or indirectly associated with them. This section will, therefore, be devoted to a brief summary of the various observations reported and theories advanced by other workers.

A. The Major Spiral. This term is used as suggested by Huskins and Smith (1935) to designate the large gyred spiral seen in many plant chromosomes at first anaphase. All large chromosomes such as those of Trillium, Tradescantia, Rhoeo, Lilium and Allium show this type of spiral. It is also indicated in many plants with small chromosomes and has been seen very clearly in wheat (R.M. Love, unpublished). Thus the spiral reported by Barenetzky has been found in the chromosomes of a great variety of plant species and may reasonably be supposed to be present in the chromosomes of all organisms. Such a characteristic structure may well be expected to be either of considerable fundamental importance itself or the physical clue to some fundamental force or forces which underlie it. A large number of workers have considered it to be of sufficient importance to warrant

rather elaborate studies. The result of these investigations has been the compilation of a fairly large number of observations, many of which are apparently contradictory, and the evolution of a number of theories concerning the origin of the spiral form.

The major spiral has been reported as developing in one of two ways depending on the organism involved. Nebel and Ruttle (1937) find the large gyred major spiral of Tradescantia developing from the widening out of a small gyred coil which develops from the waviness of the leptotene thread. Huskins and Smith (1935) find the major spiral of Trillium developing from a waviness of a magnitude more comparable to that of the mature spiral. This waviness first becomes obvious at late diplotene or early diakinesis. Any theory of origin must, therefore, take cognizance of these differences.

The earliest theory of origin advanced was proposed by Kuwada (1927). He suggested that a torsion may be set up by further contraction of the chromomema after the chromomeres come into contact with each other. This might be expected to cause first a small-gyred spiral and, when this had reached a maximum in tightness of coiling, the whole coiled thread may be thrown into a larger spiral. This, of course disagrees with the type of development reported by Nebel and Ruttle.

Apparently Kuwada later (1935) abandoned this hypothesis in favour of one which suggested that visible coiling was the

result of internal twisting in a way similar to that involved in the formation of spirals in a tendril fixed at both ends.

Darlington (1935) advanced a similar hypothesis in which he assumed that a molecular spiral sets up a torsion which results in a visible spiral which must be in the opposite direction to the internal twist.

Huskins and Smith (1935), approaching the problem from a slightly different angle, suggested that growth in thickness may occur on the outside of the chromatid and, therefore, if the half-chromatids are coiled around one another such growth necessarily occurs in a spiral. If syneresis follows the result would be a zig-zag or spiral.

The simplest hypothesis so far advanced is that proposed by Sax and Humphrey (1934). They point out that two closely associated threads compressed within an enveloping, contracting pellicle in such a way that their ends are not allowed to rotate would form a spiral of the type observed.

Of these theories only that of Sax and Humphrey could be readily modified so as to fit the developmental sequences reported by Nebel and Ruttle. It is conceivable that in this case the pellicle might, after formation of the small-gyred spiral, contract in length and expand in width to allow the coils to widen out.

All of these hypotheses assume either contraction of the chromosome or chromonema or both. That contraction of the chromosome does not occur during coiling in some cases at least was shown by Belling (1928) who found little or no difference in chromosome lengths between late diakinesis and first anaphase in Lilium and Aloe. As will be shown this is also true for Trillium and in addition to this lack of noticeable chromosome contraction the present data indicate that the chromonema instead of contracting during coiling as generally assumed actually elongates.

Any theory of coiling, to be valid, must explain not only the developmental processes involved but also account for the type of spiral observed. One such property of the completed spiral which might be expected to be of some importance in determining the most likely mode of origin concerns the direction of coiling of the chromatids and the relationships which may exist between associated threads and between homologues. A number of such studies have been carried out but unfortunately the results in some respects at least do not agree. Nebel (1932) and Sax and Humphrey (1934) find the direction of coiling to be almost random across the attachment but with a slight tendency for both arms to coil in the same direction. Nebel first reported that he could find no interstitial changes in Tradescantia but later (Nebel and Ruttle, 1936) showed that they occur occasionally. Sax (1935), in studying the direction of

coiling in Rhoeo, found rare interstitial changes. Such changes are very frequent in Trillium (Huskins and Smith 1935; Matsuura 1935, 1937; Warmke 1937). Darlington (1935) finds, particularly in Fritillaria, that the direction of coiling always reverses at the attachment and is always consistent within an arm. Randomness of coiling direction on either side of the attachment has been found in both asynaptic and synaptic Trillium by Dr. Hunter (unpublished). It has, therefore, been reported (1) that the direction of coiling on either side of the attachment is consistently opposite (Darlington), (2) completely random (Hunter) and (3) tending slightly towards being the same (Nebel, Sax and Humphrey). Furthermore, interstitial changes have been reported absent in Fritillaria, rare in Rhoeo and Tradescantia and frequent in Trillium.

Unless the first of each of these groups of observations can be proved inaccurate, either in themselves or because the numbers involved are too small, it would seem very difficult to evolve any unified hypothesis to account for such diverse cases. Sax and Humphrey (1934) point out that Darlington's hypothesis could not account for cases where the direction of coiling is the same on both sides of the attachment. Interstitial changes in direction would also be difficult to explain on a torsion theory unless, as suggested by Huskins and Smith (1935), homologues always coiled in opposite directions. If this were the case,

changes in direction would always occur at chiasmata. These authors, however, were unable to explain all changes in Trillium on this basis. Dr. Hunter (unpublished) found many changes in asynaptic material which, of course, could not be due to chiasmata. Trillium kamschaticum, which has a very low chiasma frequency, was found by Matsuura (1937) to have many more changes in direction than could conceivably be explained on the basis of chiasma influence. In fact, his analysis pointed to a direct relationship between the number of changes and chromosome length and, for this reason, he excluded chiasmata as a factor. This is probably an unjustified exclusion since his material was such that he would be unable to determine whether or not chiasmata would affect coiling. The possible rôle of these factors in relation to changes in direction in Trillium erectum will be discussed later.

B. Relational Coiling. In many publications where somatic chromosomes at late prophase or early metaphase are illustrated, it may clearly be seen that the chromatids are wound about one another in a relational coil. It is somewhat surprising that such a characteristic phenomenon has not received more attention than has so far been accorded it. Darlington (1935, 1936); Sax (1936) and Upcott (1938) have considered this problem in some detail but none of these authors has produced any definite evidence regarding the origin of

relational coiling. Darlington (1936) comes to the conclusion that "chromatid coiling at metaphase (of mitosis) seems to be developed during prophase chiefly as a result of the strain imposed on the chromatids by spiralization ..." . If his observations regarding the constancy of spiral coiling are correct then relational coiling must also show a similar constancy. He does not find this to be altogether true and explains such deviations from the expected as being due to other conditions which have not, as yet, been ascertained. On the whole, however, Darlington finds the direction of relational coiling to be the same in homologues and opposite in the two arms of the somatic chromosomes of Fritillaria and Nomocharis although certain exceptions to both statements are noted. Sax's analysis of relational coiling in Trillium grandiflorum and the "M" chromosome of Vicia faba was undertaken, not with the hope of determining the mechanism responsible, but rather as an indirect method of determining the nature of the minor (somatic) coil and the association between coiling and chiasma formation. It is interesting to note, however, that his observations on these two organisms disagree with both of Darlington's statements regarding the direction of relational coiling. He also finds, though very rarely, that the direction of the twisting may change within an arm. Upcott (1938) finds the direction of relational coiling to be random in the two arms of the nucleolar chromosome of Hyacinthus pollen grain

divisions. Furthermore, she finds no relationship between the direction of relational coiling and that of relic coiling. From this she concludes that the relic coiling is determined by the internal coiling of the previous mitosis while the relational coils are determined by new internal coils the direction of which is not in any way connected with previous coiling.

These three authors are, it would seem, all agreed in believing that there is some connection between spiralization and relational coiling. None of them, however, has indicated very clearly just what this relationship is. It is a peculiar and, perhaps, noteworthy fact that the chief point of disagreement concerning major coiling has its counterpart here in relational coiling for in both cases contradictory statements have been made regarding the direction of coiling. Concerning the direction of relational coiling two contrasting statements have been made: (1) the direction (with very few exceptions) is consistently opposite in the two arms of a chromosome and consistently the same in homologues (Darlington), (2) the direction is random in the two arms and may change, though very rarely, within an arm (Sax, Upcott) and the direction is also random between homologues (Sax). An hypothesis concerning the origin of relational coiling, therefore, must surmount the same type of obstacle as encountered by a hypothesis of spiral coiling in that unless one or the other of these sets of observations

is inaccurate it must explain very diverse results.

In subsequent sections some preliminary data on relational coiling in the first pollen grain division of Trillium erectum will be given and analyzed, in so far as the number of observations permits analysis, with respect to the work which has just been reviewed.

C. The Tertiary Split. Mention has already been made of this cleavage in reference to the structure of first anaphase chromosomes. As will be seen later, the time of its occurrence may have some bearing on the problem of relational coiling and, therefore, the question of its reality may be of some importance. Darlington (1932, 1935, 1937) does not believe that it occurs before the resting stage prior to pollen-grain division. Sax and Humphrey (1934) find no evidence for the tertiary split at first anaphase in Tradescantia. On the other hand, Huskins and Smith (1935) and Warmke (1937) report this split as being very obvious at first anaphase in Trillium and apparently some trace of it may be seen slightly earlier. Nebel and Ruttle (1937) find the first metaphase bivalents of Trillium and Tradescantia to be sixteen-partite. They, therefore, agree with Huskins and Smith and Warmke that a split occurs about first metaphase but consider the tertiary split of these authors to have occurred at metaphase of the previous mitosis. McClintock

(unpublished) has shown octopartite structure very clearly in the pachytene chromosomes of Zea Mays.

D. The Matrix and Pellicle. Several authors (Kaufman, 1926; Nebel, 1932; Koshy, 1933; Marshak, 1936) have described the chromosome as consisting of an achromatic and chromatic part. The former is the discontinuous phase while the latter is the continuous chromonema. Most workers consider this discontinuous phase to be a matrix which is produced by, and in which, the chromonema lies. Darlington, however, is not inclined to accept the reality of the matrix. He says (1937, p. 566): "The matrix is the whey, which is separated from the curd by fixation ..... In its use to fill a morphological and mechanical rôle --- the word matrix is nothing more than a myth". While it would seem "hair-splitting" tactics to divorce matrix and pellicle, yet Darlington (1935b) states that: "There are several grounds -- chiefly non-morphological -- for assuming that the chromosome thread has some sort of a pellicle". Sax and Humphrey (1934) consider that the presence of a hyaline area about the chromonema is sufficient evidence of the reality of the pellicle and, therefore, attach to it a considerable importance as a factor in the mechanical alterations of the chromonema. Kuwada (1935) states that the matrix in contracting completes the spiral. He thus combines pellicle and matrix under the one name. Coleman and Hillary

(unpublished), using a modification of the technique of Caspersson, have determined the presence of a non-nucleic-acid-containing substance, digestible by a proteolytic enzyme, surrounding the nucleic-acid-containing chromonema.

E. The Attachment Region. It is obvious from all cytological observations that each chromosome has a differentiated region situated at a definite and (for any one chromosome) constant region which plays some part in anaphase separation. Numerous authors have called this region the attachment or insertion region because of its apparent connection with the "spindle fibers". Under ordinary conditions of fixation and staining, the attachment appears as a constriction but under special treatments (Nawaschin, 1912; Trankowsky, 1930) it may appear as a round dot about 0.2μ in diameter. Darlington (1936) has called this body the centromere and has considered it to be a somewhat specialized chromomere with special properties of attraction and repulsion. Schrader (1939) has pointed out that this term has previously been used by Waldeyer (1902) to denote the neck region of the sperm. Sharp (1934) describes the attachment as being a region of definite length in some cases and in others to be homogeneous except for the presence of a number of centrally placed kinetic bodies. It has been well proven that fragments lacking it cannot behave normally and are generally lost. Certain cases in Zea (McClintock, 1932) however, indicate that fragmentation may occasionally

occur through the spindle attachment in which case both resulting parts behave normally. Upcott (1938) has explained a number of abnormalities in Tulipa on a similar basis. She assumes that occasionally the cleavage of the centromere may occur in a different plane than that in which it normally does and thus gives rise to numerous abnormal distributions of chromosome arms. Huskins and Smith (1935) found the attachment in Trillium extending out as a loop from each side of the metaphase bivalent but they were unable to determine whether the entire region was differentiated or whether a centrally placed body was acting to pull out portions of the chromonema on either side. It was noted, however, that this loop region often stained less deeply than the rest of the chromonema. Further data concerning the properties of this loop have been obtained and a discussion of this point will be given later.

MATERIAL AND METHODS.

During the months of September or October of the years 1932, 1935, 1936, 1937, and 1938, collections of *Trillium erectum L.* were made at Ste. Agathe, Quebec. After collection the corms were placed in flats or cans and subjected to various temperatures as represented by the greenhouse, controlled temperature chambers, the refrigerator and the laboratory. Preliminary surveys showed that the pollen mother cells were, for the most part, at leptotene or earlier when collected. They were, therefore, allowed to remain under the conditions chosen until smeared at appropriate stages. Data from material representing all five years have been considered in this study although the writer has been concerned with the handling of the last three years' material only. Table I gives an outline of the history of the most important material used in the present investigation.

Two methods of preparation have been used during the course of these studies. Asynaptic material was mascerated in iron-aceto-carmine without previous fixation and made permanent by McClintock's method. The best preparations in all other material were made by smearing fresh anthers, dessicating for 20-30 seconds (with the exception of pollen grain division slides which were not dessicated) and fixing in LaCour's 2BD for 2 hours. The slides were then washed in water, bleached in hydrogen peroxide and stained in crystal

TABLE I.

Outline of History and Treatment of Material.

Material	Serial or Slide Nos.	Kept During Meiosis	Temperature	Duration of Treatment
*1. Synaptic		Green-house	Approx.15°C	Oct. - Dec. 1932.
**2. Asynaptic Type (1) Type (2)	57	Temperature Chambers	8° - 16°C	Oct. {Dec. 15 Dec. 30 1935
3. Desynaptic	NW-4L-1-15	Temperature Chamber No.4	12°C	Nov.24 - Nov.27, 1936-37
4. Synaptic	58-2E-1	Temperature Chamber No.2	4°C	Sept.30 - Jan.10, 1937-38
5. Synaptic	58-4E-1	Temperature Chamber No.4	12°C	Sept.30 - Dec.16, 1937
6. Synaptic	58-5H-3 58-5d-3	Temperature Chamber No.5	16°C	Sept.30 - Nov.23, 1937
7. Synaptic,non-coiled	58-6d-3	Temperature Chamber No.6	20°C	Sept.30 - Oct.29, 1937
8. Synaptic	65-L2-a	Laboratory	20° - 22°C	Oct.5 - Nov. 2, 1938
9. Synaptic,non-coiled	65-C7-c	Conservatory	Approx.20°C	Oct.4 - Nov. 2, 1938
10. Pollen-Grain Division	65-M 65-P	Laboratory	23°C 15° - 22°C	Oct.5 - Nov.25, Sept.14 - Nov.30 1938
11. Pollen-Grain Division	NW-5E-2	Temperature Chamber No.5	15°C	Nov. 24 - Dec.16, 1936
12. Pollen-Grain Division	63-RC	Refrigerator Laboratory	2° - 3°C 18° - 22°C	Oct. - Jan.15 Jan. 15 - Jan.20 1938-39

Note: The 1936 material was kept in the refrigerator from Oct. 30 - Nov. 24.

* Reported on by Huskins and Smith (1935) and Hunter, A.W.S. (unpublished, Ph.D. Thesis).

** Hunter.

violet as described by Huskins and Smith (1935). In my own material, this was modified to the extent that fixation time was increased to 6-8 hours and the staining time has been reduced to 10-20 seconds.

Observations were made with a Zeiss 1.5 mm., 1.3 N.A. objective combined with 7x, 15x and 20x oculars. All figures used for measurement were drawn with the camera lucida at original magnifications of 4000x and 3700x. Those which have herein been reproduced for illustration have been reduced to 2000x in photographing. Measurements have been made from camera lucida drawings and checked, in so far as possible, with a no.3 micrometer eye-piece calibrated for the 1.5 mm. objective.

OBSERVATIONS.

1. General.

Meiosis.

A. The First Division. The present material has not been found very satisfactory for studying meiotic prophase stages. Such observations as have been made, however, are essentially in agreement with those reported by Huskins and Smith (1935). All preparations which show the leptotene stage were examined in order to determine whether or not the chromosome is split at this stage. In so far as could be determined, there is little evidence that they are other than optically single. It was noted, however, that the chromomeres were occasionally dumb-bell shaped which must be taken to indicate some degree of doubleness. Either a semi-healing of a previous split has occurred or a new cleavage is developing.

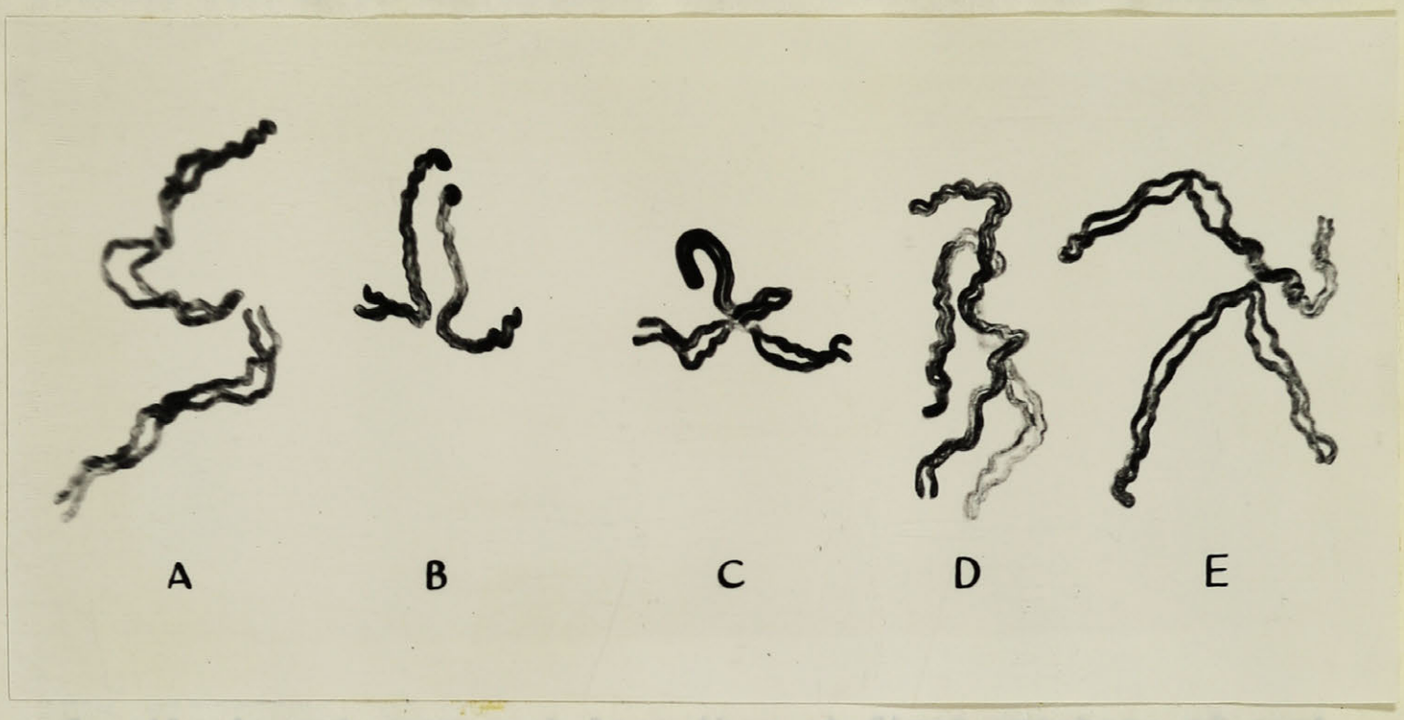
No evidence for the view that the chromomeres are actually gyres of a fine spiral or loops between turns of two intertwined threads could be found. The distance between them compared to their size is too great for either interpretation to be justified. From zygotene to early diplotene they apparently move closer together since the distance between them decreases and at late diplotene they may, under some conditions, appear as the turns of a small gyred spiral the bars of which, however, traverse the two strands.

Huskins and Smith state that a slight contraction probably occurs in the chromosome between leptotene and pachytene. An attempt was made to test the validity of this statement by measuring the distance between chromomeres at various stages. The small number of satisfactory cells available and the mechanical difficulty of making such measurements rendered the few results obtained quite inconclusive. It is clear, however, that whatever change in length occurs must be very slight and there seems some reason to believe that a slight elongation is taking place between leptotene and zygotene (cf. Belling, 1931).

Though it has not been shown in most of the text figures, some indication of the tertiary split was apparent in most first anaphase chromosomes examined. In a few cases its presence was indicated somewhat earlier. In one case (Text fig. 1) where major coiling was lacking at first anaphase, the tertiary split was so obvious as to make any other interpretation impossible.

Some observations have been made on the physical appearance of the attachment at first metaphase and anaphase. As indicated in text figure 14, it is apparently a region of 2 - 3μ in length. Within this regions there seems to be a certain amount of differentiation in regard to forces of attraction, the distal portions being very closely associated or even fused with the corresponding portions of the sister attachment while the central portion shows an apparent

lack of attraction. A similar situation may also be found in the chromosomes of pollen-grain division (Text fig. 18).



Text fig.1. Chromosomes at one pole of a first anaphase cell from material 65-C7-c, showing a minor waviness and a very distinct tertiary split. Note that there is no major coiling.

B. <u>The Second Division.</u> There is normally no interkinesis in Trillium so that the chromosomes pass directly from first anaphase to second metaphase without material change in structure. The matrix seems to increase slightly in chromaticity since it is much more difficult to obtain a clear image of the chromonema. The number and size of the coils, and the lengths of the chromosome

and chromonema are approximately the same as at first anaphase (Text fig. 7). In most cases the tertiary split is very clear, particularly at second telophase.

Pollen Grain Division.

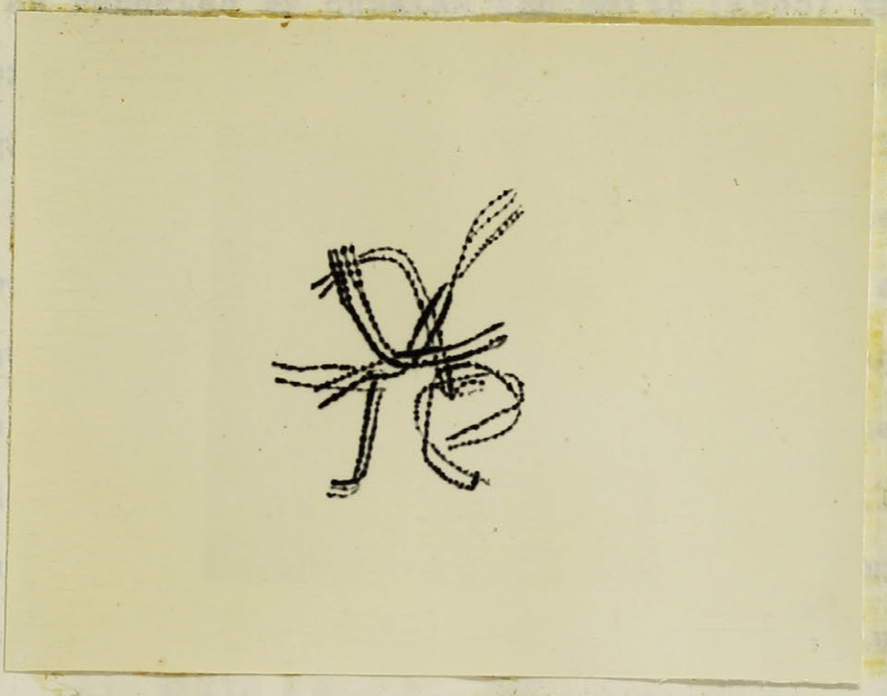
It has, so far, been found quite impossible to trace the chromosomes through resting stage but in some regions of many metabolic nuclei, they seem to be lying in the position in which they entered resting stage and appear to retain about the same number and size of coils as at second telophase. That there has been little movement is shown by the fact that early prophase chromosomes are bent at the attachment as they were when passing to the poles at second anaphase and the number of coils is not very much less than at first anaphase of equivalent material. M.B. MacKenzie (unpublished) has analyzed several cells from this aspect and finds the number of gyres per five chromatids to be 40 - 50 as compared to 50 - 60 at first anaphase (Text fig. 16). Since it is impossible to follow the chromosomes through resting stage with any degree of certainty there is obviously a possibility that the relic coil is not the remains of the major coil. Any other interpretation, however, seems somewhat illogical particularly in view of the fact that the relic coil is unravelling during prophasic contraction.

At early prophase under suitable conditions the chromosomes may be seen to be double and each chromatid has a beaded

or chromomeric structure similar to that observed in prophase of meiosis, except that the chromomeres are closer together than at leptotene or zygotene. This may well be due to the greater length of the meiotic thread since MacKenzie finds the five chromosomes of early prophase of pollen-grain division to total about 200μ in length while the pachytene chromosome length estimated from text-figure 3 of Huskins and Smith (1935) is upwards of 300μ . There seems little reason to believe that these "beads" represent a fine spiral although they do give this appearance at late prophase and early metaphase except where it can be seen that each chromatid is double. In this case the chromomeres are too small relative to the distance between them to make their interpretation as gyres of a spiral very probable.

Late prophase and early metaphase chromosomes always show some indication of a four-partite structure and, in some cases, it is so clear as to make any other interpretation impossible (Text fig. 2; Plate IV, fig. 14).

As soon as the doubleness of the chromosomes becomes apparent it may be seen that the chromatids are more or less relationally coiled. This coil does not seem to be regular since relatively long portions and occasionally whole chromosomes at mid-prophase may show no twisting. The number of twists is reduced during prophase contraction but they may not be eliminated entirely even at full metaphase.



Text fig. 2. An early metaphase nucleus at first pollen-grain division showing the four-partite condition and the chromomeric structure of the component strands.

No somatic spiral is evident prior to late metaphase at which time the development of the matrix has reached the point where it obscures internal structure. In many preparations, however, an indication of a spiral thread may be seen within the chromosome and in a few cases a spiral can be followed for several gyres (Plate IV, fig. 15). Except for size and the number of turns this spiral seems to be similar in every way to the major coil of meiosis.

2. The Development of the Major Spiral.

As reported by Huskins and Smith (1935) the major spiral of Trillium erectum develops gradually from a more or less regular waviness which may first be seen at late diplotene or early diakinesis. During the present investigation an effort has been made to determine what length changes, if any, occur in the chromosome and chromonema during this period of development. To this end a number of measurements were made at diakinesis and first anaphase as well as a few at second anaphase. Both chromosome and chromonema lengths were obtained on several hundred chromosomes including both complete cells and any miscellaneous chromosomes which were suitable for measurement. As indicated by Tables II-VI these were grouped according to treatments so that only comparable values are compared. Text figs. 3-11 illustrate chromosome complements typical of the materials not previously illustrated elsewhere.

The methods of measurement employed were necessarily somewhat crude and the values obtained are, therefore, liable to considerable error. Every effort has been made, however, to reduce this error to a minimum. All measurements have been checked several times in order to eliminate any errors in the reading of figures and, in so far as possible, more than one method of obtaining values has been used. Chromosome lengths and widths have been obtained both from

TABLE II.

Diakinesis and Anaphase Chromosome and Chromonema Lengths from Synaptic Material (1)*.

Diakinesis.

Chromosome: A		B		C		D		E		Totals	
Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length
-	-	12.7	17.6	10.6	12.9	13.6	23.5	15.5	30.4	52.4	84.4

Anaphase I.

Chromosome: A				B				C				D				E				Totals			
		Ch'ma. Length				Ch'ma. Length				Ch'ma. Length				Ch'ma. Length				Ch'ma. Length				Ch'ma. Length	
Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula
-	-	-	-	12	12.5	28.7	49.7	9	10	20.7	38.1	12	13	24.3	40.8	16	16.4	45.4	73.8	49	51.9	119.1	202.4

Diameter of Coil = 1.5μ

* Obtained by measuring figures in Huskins and Smith (1935).

TABLE III.

Chromosome and Chromonema Lengths at Diakinesis and Anaphase of Material from Slide 58-4E-1.

Diakinesis.

Chromosome:	A		B		C		D		E		Totals	
	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length
*1.	11.0	13.7	10.2	13.5	8.2	10.0	10.0	12.7	14.0	20.0	53.4	69.9
*2.	10.6	11.0	10.0	13.2	7.5	11.3	11.2	13.0	13.7	20.0	53.0	68.5
*3.	10.0	13.0	10.0	12.0	6.3	9.0	8.8	12.0	12.5	16.0	47.6	62.0
*4.	9.0	10.7	10.7	12.7	10.0	12.2	13.2	16.3	16.3	19.0	59.2	70.9
5.	11.3	12.1	10.0	12.0	7.5	9.0	-	-	13.8	16.9	-	-
*6.	9.2	11.0	10.5	12.7	10.0	12.2	13.0	16.0	16.5	19.0	59.2	70.9
Mean	10.2	11.8	10.2	12.7	8.2	10.6	11.2	14.0	14.5	18.5	54.3	67.6

* Complete nuclei. No.6 was drawn by Mr. H.B. Newcombe.

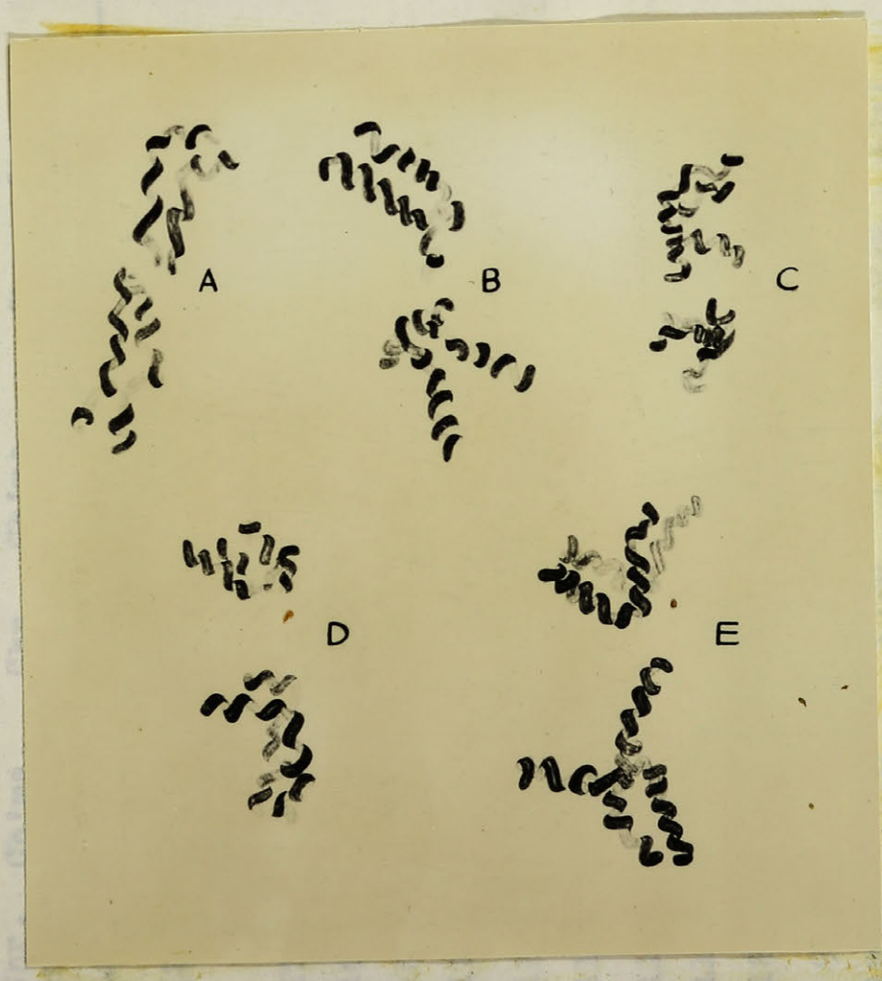
Anaphase I.

Chromosome:	A				B				C				D				E				Totals			
			Ch'ma. Length				Ch'ma. Length				Ch'ma. Length				Ch'ma. Length				Ch'ma. Length				Ch'ma. Length	
	Gyres	Chr. Length	Measured in one plane	Calc. from coil formula	Gyres	Chr. Length	Measured in one plane	Calc. from coil formula	Gyres	Chr. Length	Measured in one plane	Calc. from coil formula	Gyres	Chr. Length	Measured in one plane	Calc. from coil formula	Gyres	Chr. Length	Measured in one plane	Calc. from coil formula	Gyres	Chr. Length	Measured in one plane	Calc. from coil formula
*1.	7	10.0	16.2	35.1	8	10.0	20.8	34.8	6	8.5	14.7	30.1	9	12.5	20.5	43.5	13	16.0	27.2	65.8	43	57.0	99.4	209.3
*2.	6	10.0	11.6	30.6	8	11.5	14.5	39.8	7	8.8	13.0	34.4	8	11.5	16.5	37.2	11	15.0	25.9	55.0	40	56.8	81.5	197
3.	7	7.5	15.0	34.0	9	8.8	14.0	43.4	5	6.3	13.0	24.6	8	9.2	13.5	38.6	12	12.5	20.5	56.0	41	44.3	76.0	196.6
4.	7	7.5	12.0	34.0	9	9.2	17.0	43.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	7	8.7	15.0	34.4	7	8.7	12.0	34.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	7	8.9	13.9	33.6	7.7	9.6	15.6	39.1	6	7.8	13.6	29.7	8.3	11.1	13.8	39.7	12	14.5	24.5	58.9	41	51.9	84.4	201.0

Diameter of Coil = 1.5μ

* Complete nuclei

Text fig. 3. Two diakinesis chromosome sets from slide 58-4E-1.



Text fig. 4. A first anaphase complement from slide 58-4E-1.

TABLE IV A.

Diakinesis Chromosome and Chromonema Length from Slide 58-5d-3.

Chromosome:	A		B		C		D		E		Total	
	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length
*1.	12.0	15.4	13.7	16.4	12.5	15.7	14.2	16.7	21.4	24.3	73.8	88.5
2.	-	-	14.5	15.5	-	-	-	-	-	-	-	-
*3.	12.5	14.5	11.3	13.0	8.8	13.0	16.3	17.5	22.5	27.1	71.4	85.1
4.	10.0	13.5	12.5	17.0	-	-	16.3	20.5	17.5	34.5	-	-
*5.	10.0	12.0	12.5	16.0	8.2	10.5	14.4	16.0	18.8	21.2	63.9	75.7
*6.	12.5	13.5	10.0	11.6	9.0	10.0	15.4	18.0	18.5	20.0	65.4	73.1
*7.	12.0	20.0	13.6	19.0	11.7	12.7	15.0	19.0	20.0	30.0	72.3	100.7
*8.	11.2	12.0	12.0	16.3	10.7	13.7	14.6	17.0	17.6	18.3	66.1	77.3
*9.	11.0	12.2	12.0	14.1	9.0	12.7	12.0	14.6	17.5	22.2	61.5	75.8
*10.	11.2	13.7	10.0	12.7	8.5	11.7	12.4	14.4	16.3	23.2	58.4	75.7
*11.	12.0	13.4	10.5	15.0	7.5	10.2	14.1	18.0	17.6	19.0	61.7	75.6
*12.	9.0	10.7	10.7	12.2	7.5	9.2	10.7	12.2	17.0	19.0	54.9	63.3
*13.	12.0	16.0	15.0	17.0	10.0	13.7	13.8	24.4	17.0	25.3	67.8	96.4
*14.	11.5	12.7	11.5	15.0	8.5	12.9	12.4	14.6	20.7	24.0	64.6	79.2
*15.	16.0	19.0	16.1	17.0	14.1	17.5	16.6	19.0	20.0	25.0	82.8	97.5
Mean.	11.6	14.2	12.4	15.4	9.6	12.6	14.1	17.2	18.7	23.8	66.4	83.2

* Complete nuclei. Nos. 6-15 were drawn by Mr. Newcombe.

TABLE IV B.

Anaphase Chromosome and Chromonema Lengths from Slide 58-5d-3.

Anaphase I.

Chromosome:	A				B				C				D				E				Totals			
	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula	Gyres	Chr. Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula
*1.	7	11.4	20.3	35.7	9	13.2	18.9	45.0	8	10.9	17.4	37.6	9	13.7	20.4	40.9	12	17.1	34.8	60.4	45	66.3	111.8	219.6
*2.	7	9.2	14.5	32.4	9	20.0	20	43.7	6	8.1	13.0	28.0	8	11.3	16.5	32.6	12	17.5	22.5	58.5	42	57.4	86.5	195.2
3.	7	11.0	16.5	33.2	9	16.0	22	43.5	7	11.0	14.5	33.4	9	14.0	19.0	42.4	13	16.5	28.0	59.7	45	68.5	110	212.2
4.	8	11.0	17.0	37.3	-	-	-	-	-	-	-	-	-	-	-	-	14	19.0	24.5	64.5	-	-	-	-
Mean	7.2	10.6	17.1	34.6	9	13.5	20.3	44	7	10	14.9	33	8.9	13	18.6	38.6	12.7	17.5	27.4	60.8	44.8	64.6	98.3	211.0

Diameter of coil = 1.5 μ

Anaphase II.

Chromosome:	A				B				C				D				E				Totals			
	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula	Gyres	Chr.Length	Ch'ma. Length. Measured in one plane	Ch'ma. Length. Calculated from coil formula	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula	Gyres	Chr.Length	Ch'ma. Length Measured in one plane	Ch'ma. Length Calculated from coil formula
*1.	6	8.7	13	30.2	7	10	20	34.8	6	8.7	14	30.2	7	11.2	18	35	12	16.2	25	59	38	54.8	90	189.2
*2.	7	7.9	15	28	8	9.1	16.2	32.0	7	6.7	14.5	27.5	8	9.4	16.7	32.0	12	13.3	24	47.5	42	46.4	86.4	167.0
*3.	7.5	10.8	17	35.2	8	12.2	20	37.8	7	9.0	16.0	32.6	7	11.0	18.0	33.4	11.5	16.2	27	54.0	41	59.2	98	193.0
Mean	6.9	9.1	15	31.1	7.7	10.4	18.7	34.9	6.6	8.1	14.8	30.1	7.1	10.5	17.6	33.5	11.7	15.2	25.3	53.5	40.0	53.3	91.4	183.1

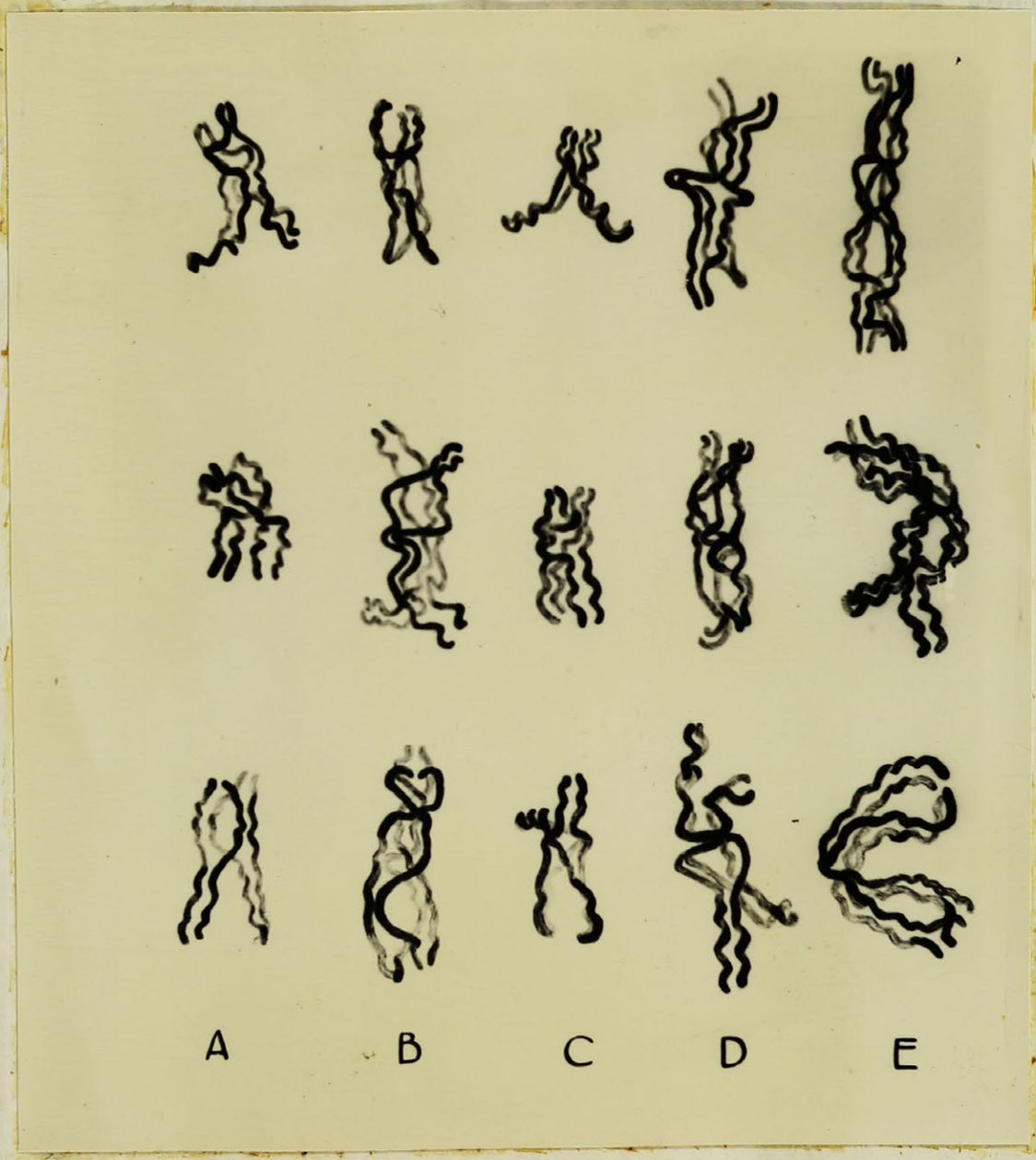
Note: The diameters of the coils at second anaphase are as follows for each of the three cells: 1. 1.5μ, 2. 1.2μ, 3. 1.4μ.

* complete nuclei.

direct measurements made with a micrometer ocular and from calculations based on camera lucida drawings. Diakinesis chromonema lengths were calculated from measurements of the camera lucida drawings, the length of the strand or strands which came nearest to lying in one plane being taken to be the most accurate and is the value given in the tables. A similar method of measuring the fully coiled anaphase chromonema does not, of course, give the true length of the thread but it does supply a valuable basis for further calculation. By constructing a pipe-cleaner model to scale it was found possible to estimate the relationship between the true length of a spiral thread and its one plane image. The model was placed under a glass drawing board and its image traced. The length of the tracing was determined and compared with the true length of the pipe cleaner. In this way it was found that one-plane measurements involving about fifty gyres would be about half the true length of the thread. A fair estimation of the chromonema length at anaphase could, therefore, be obtained by doubling the values obtained from a one-plane measurment of drawings. A second method which is slightly more accurate, at least theoretically, is to use the formula for a spiral coil:

$$d = n\sqrt{p^2 + 4\pi^2 r^2}$$

where d = the length of the thread, n = the number of gyres, p = the pitch, and r = the radius of the coil. This involves the determination of three variables; the number of gyres, the chromosome length, and the diameter of the coil.



Text fig. 5. Three diakinesis chromosome sets from slide 58-5d-3.

The first can be determined with considerable accuracy. Most of the error is introduced through measurements of length and width. This is reduced, however, by careful checking and by using both the micrometer eye-piece and drawings and by avoiding chromatids which are not lying flat. Tables II-VI were constructed from data obtained by these means.

Text fig. 6. A first anaphase complement from slide 58-5d-3.

Text fig. 7. A second anaphase complement from slide 58-5d-3.

It is obvious from these data that elongation of the chromonema occurs between diakinesis and anaphase while the length of the chromosome changes little or not at all. Any change which does occur in the latter seems to be in the direction of contraction rather than elongation and, in any case, is minute when compared to the change in chromonema length. An analysis of these data in relation to the problem of coiling will be found in the discussion.

TABLE V.

Chromosome and Chromonema Lengths at Diakinesis and Anaphase of Material from Slide 65-L2-a.

Diakinesis.

Chromosome:	A		B		C		D		E		Totals	
	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length
1.	12.0	16.0	12.0	17.5	12.4	15.0	17.5	20.0	24.0	31.0	77.9	99.5
2.	10.0	18.0	14.0	17.0	12.4	17.0	16.0	25.0	24.0	31.0	76.4	108.0
3.	12.7	15.0	18.0	22.0	11.7	16.0	17.0	22.0	23.0	31.0	82.4	106.0
4.	12.0	17.0	15.0	23.0	11.5	18.0	17.0	24.5	18.5	27.5	74.0	110.0
Mean	11.7	16.5	14.8	19.9	12.0	16.5	16.9	22.9	22.4	30.1	77.8	105.9

Note: All chromosomes were drawn by Mr. Newcombe.

Anaphase I.

Chromosome:	A				B				C				D				E				Totals			
			Ch'ma. Length				Ch'ma. Length				Ch'ma. Length				Ch'ma. Length				Ch'ma. Length				Ch'ma. Length	
	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula	Gyres	Chr. Length	Measured in one plane	Calculated from coil formula
1.	10.0	9.5	18	39.0	12.5	13.0	22.5	49.3	9 10	16.0	35.7		11.0	13	25.0	44.0	18	17.5	35	70.0	60.5	63.0	116.5	238.0
2.	9.5	11.7	18	32.8	11.5	12.5	22.0	38.7	9 12	19.0	31.3		12.0	17	24.0	42.8	16	23.0	33	55.7	58.0	76.2	116.0	201.3
Mean	9.7	10.6	18	35.9	12.0	12.7	22.3	44.0	9 11	17.5	33.5		11.5	15	24.5	43.4	17	20.2	34	62.8	59.2	69.5	116.3	219.6

Diameter of Coil = 1.2μ

Text Fig. 8. A diakinesis nucleus from slide 65-L2-a.

Text fig. 9. A second metaphase nucleus from slide 65-L2-a.

TABLE VI.

Chromosome and Chromonema Lengths at Pre-metaphase and Anaphase Stages of Material from Slide 58-6d-3.

Pre-metaphase.

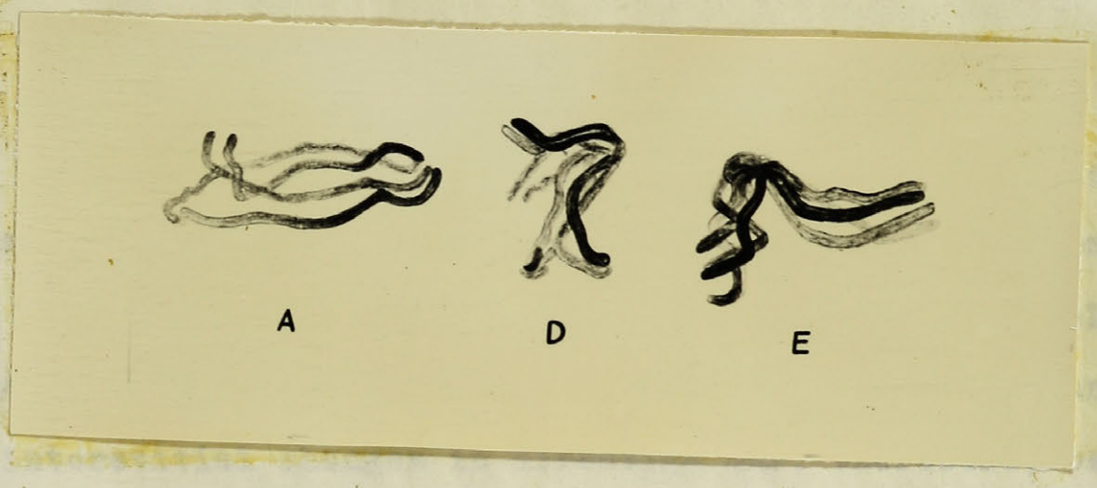
Chromosome:	A		B		C		D		E		Totals	
	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length
*1	-	-	12.8	13.4	-	-	17.5	18.8			-	-
**2	15.8	17.1	17.5	19.2′	15.0	16.7′	20.0	21.0	26.5	30.0	94.8	103.0

* Early diakinesis.
** Late diakinesis.
′In these two chromosomes, chromonema lengths could not be determined accurately; these values were, therefore, calculated by adding to the chromosome lengths the mean difference between the chromosome and chromonema lengths of the other chromosomes in the cell.

Anaphase I.

Chromosome:	A		B		C		D		E		Totals	
	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length	Chr. Length	Ch'ma. Length
1.	24.1	25.8	22.6	24.2	16.4	18.9	24.3	24.3	36.3	37.5	123.7	130.7
2.	16.7	17.4	25.0	26.1	17.5	18.7	30.0	32.0	36.0	37.5	125.2	131.7
Mean	20.4	21.6	23.8	25.1	16.9	18.8	27.1	28.1	36.2	37.5	124.4	131.1

Note: The chromosomes of group (1) are from one cell with the exception of B. Group (2) consists of miscellaneous chromosomes.



Text fig. 10. Chromosomes A,D and E of a late diakinesis complement from slide 58-6d-3. Note lack of major coiling.

Text fig. 11. A first anaphase complement, from slide 58-6d-3, showing lack of major coils.

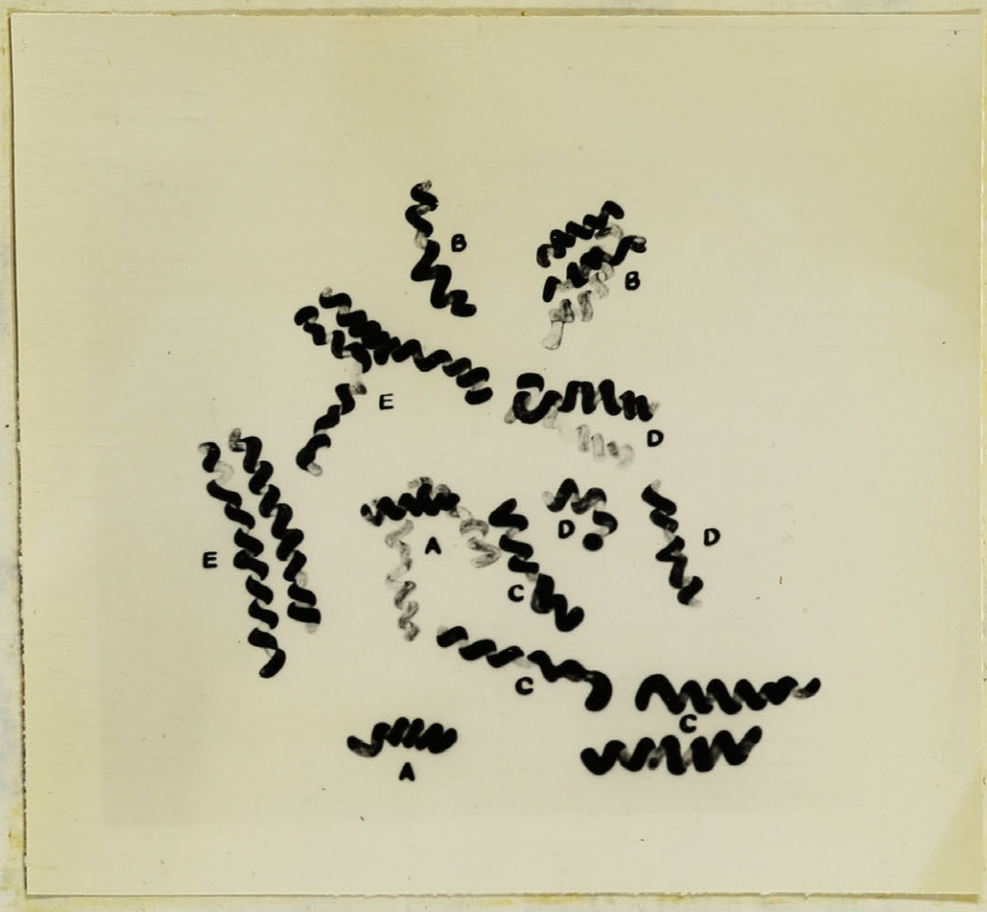
3. The Direction of Coiling.

Huskins and Smith (1935) found that the direction of coiling may change anywhere along the length of the chromonema. From such observations as they made, they also believed that homologues commonly coiled in opposite directions and that interstitial changes in direction may, therefore, be due to chiasmata. They pointed out, however, that this hypothesis could not explain all observed changes. Dr. A.W.S. Hunter (unpublished) found the direction of coiling to be more nearly random between homologues and perfectly so in the case of asynaptic material.

During the present study, data from several other sources have been added to those of Huskins and Smith and Hunter and the whole analyzed in an attempt to determine the nature and cause of changes in direction. All analyses are based on anaphase chromosomes since they are more easily interpreted at this stage. For convenience the materials used may be listed as follows:

(1). Synaptic material, analyzed by Huskins and Smith and Dr. Hunter, kept at about 15°C in the green-house during the pre-anaphase stages of meiosis.

(2). Desynaptic material (Text fig. 12) which underwent meiosis at 9°C in a temperature chamber (Slide NW-4L-1-15).



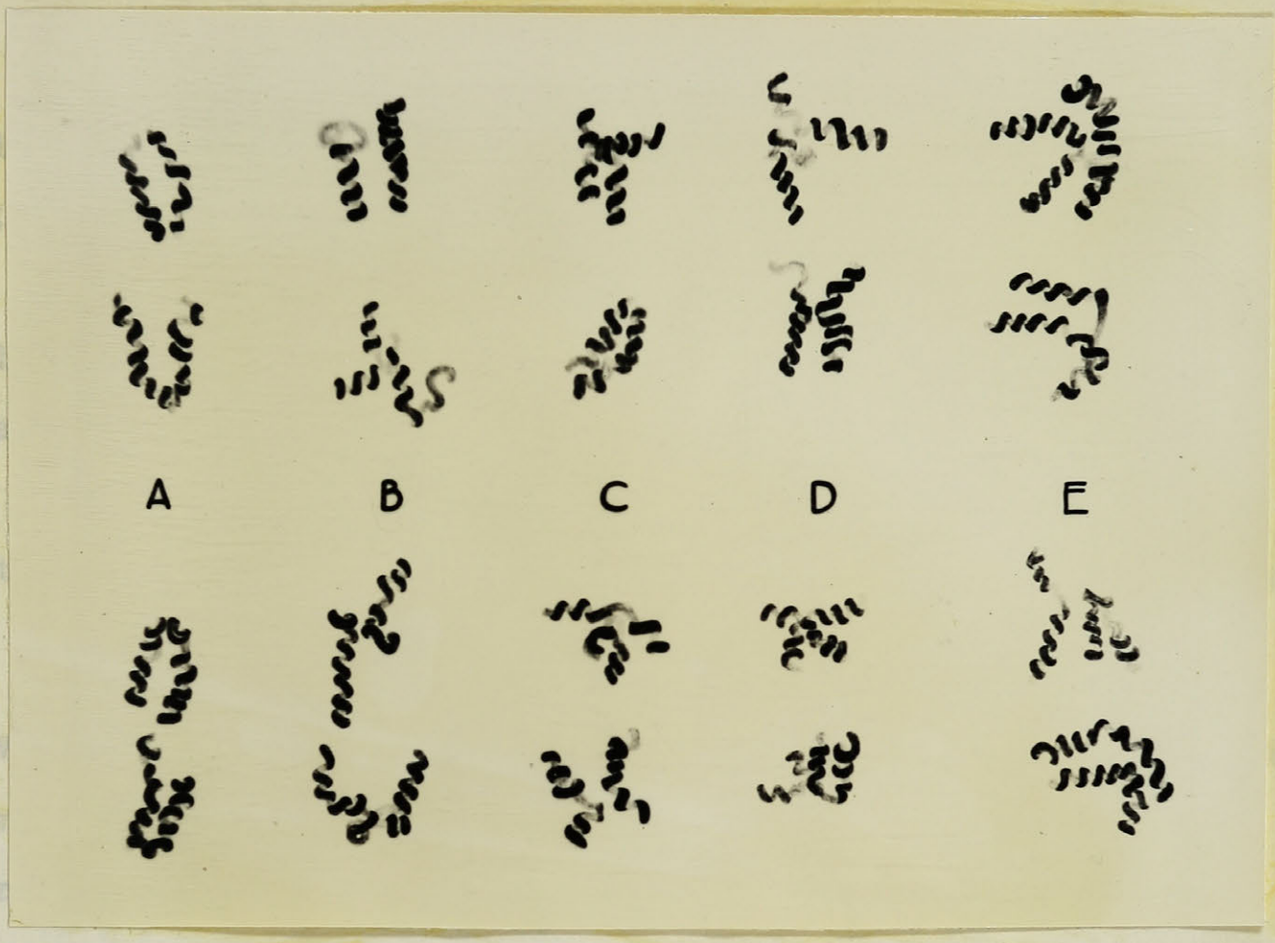
Text fig. 12. A nucleus from slide NW-4L-1-15 showing desynapsis of homologues and separation of sister strands.

(3). Two types of asynaptic material obtained by Dr. Hunter.

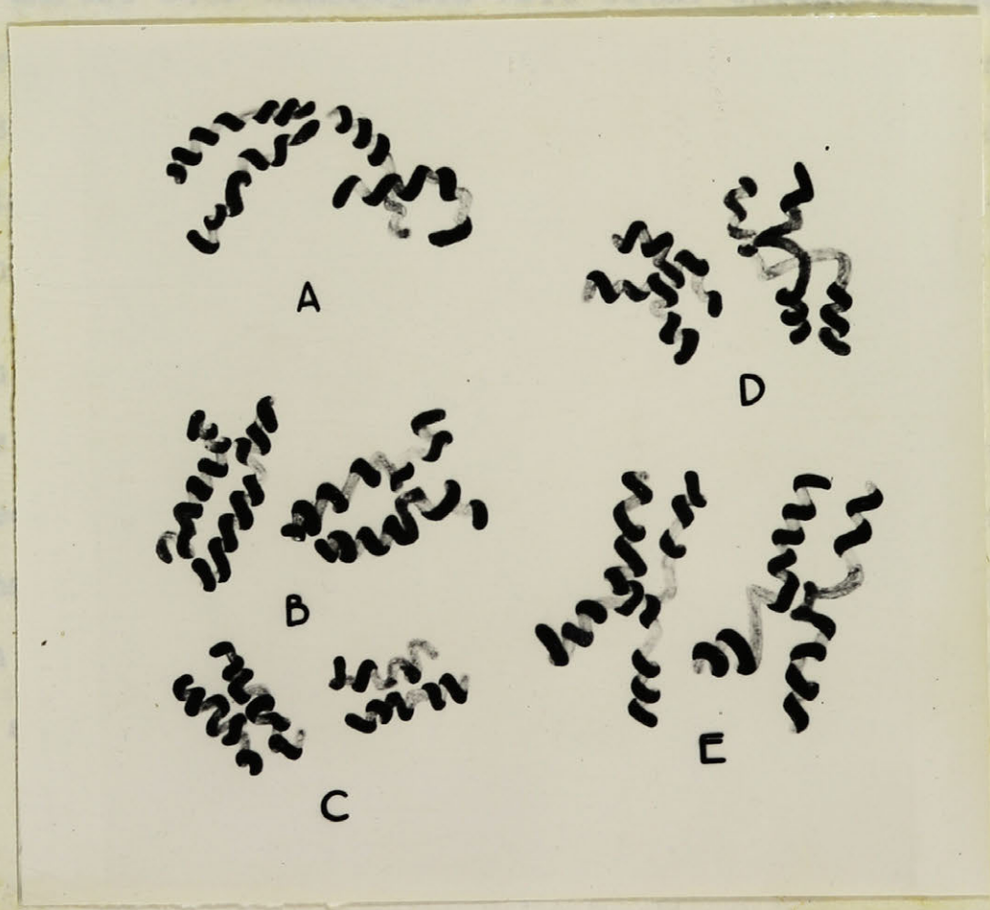
a. Type (1) showed asynapsis between homologues but sister strands were closely associated.

b. Type (2) showed both asynapsis of homologues and wide separation of sister strands.

(4). Synaptic material (Text fig. 13) which underwent meiosis at 4°C in a temperature chamber (Slide 58-2E-1).



Text fig. 13. The chromosome complements of two first anaphase cells from slide 58-2E-1.



Text fig. 14. A first anaphase chromosome complement from slide 58-5H-3. Note the attachment regions in chromosomes B, C and E.

(5). Synaptic material (Text fig. 4) which was kept in a temperature chamber at 11° - 12.5°C during meiosis (slide 58-4E-1).

(6). Synaptic material (Text figs. 6,7,14) kept at 16°C in a temperature chamber during meiosis (slides 58-5d-3 and 58-5H-3).

(7). Synaptic material (Text fig. 9) which passed through meiosis at 20 - 22°C in the laboratory (slide 65-L2-a).

The data from these materials are given in Tables VII-XIII. The number of gyres per chromatid is given as the mean for the four homologous chromatids of a bivalent. The number of changes in direction per chromosome is the total for all four chromatids. No chromosomes were used in this analysis unless both homologues were clear enough for accurate interpretation. Most of the anaphase chromosomes on slides 58-5d-3 and 65-L2-a were very loosely coiled which made it impossible to determine the number of changes in direction with complete accuracy.

In order to analyze the changes in direction in these materials with reference to the possible effect of chiasmata, it was necessary to determine the chiasma frequency of the various materials as accurately as possible. These data are given in Table XIV. The following explanatory statements serve to show exactly how the various values were obtained:

TABLE VII.

Number of Gyres and Changes in Direction in Anaphase Chromosomes from Material (1)*.

Chromosomes	Complete Nuclei						Miscellaneous Chromosomes										Mean	
	1		2		3		4		5		6		7		8			
	No.Gyres	No.Changes	No. Gyres	No.Changes	No.Gyres	No.Changes	No.Gyres	No.Changes	No.Gyres	No.Changes	No.Gyres	No.Changes	No.Gyres	No.Changes	No.Gyres	No.Changes	No.Gyres	No.Changes
A.	10.5	6	10	7	10	2	11.0	6	9.5	4	10.0	3	10.5	7	10.5	6	10.2	5.1
B.	12.0	13	12	11	10	2	11.0	11	10.5	9	11.0	7	-	-	-	-	11.1	8.8
C.	9.0	5	9	11	8	7	9.0	9	9.0	11	-	-	-	-	-	-	8.8	8.6
D.	11.5	10	12	5	12	9	11.5	5	11.0	8	12.0	10	-	-	-	-	11.6	7.8
E.	15.0	13	16	12	17	10	16.0	18	16.0	10	16.5	13	16.0	10	-	-	16.1	12.3
Total	58.0	47	59	46	57	30	58.5	49	56.0	42							57.8	42.6

* See p. 51 of Text.

TABLE VIII.

Number of Gyres and Changes in Direction in Anaphase Chromosomes from Material (2) - Slide NW-4L-1-15.

Chromosome	Complete Nuclei				Mean	
	1		2			
	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes
A	6.0	6	6.0	3	6.0	4.5
B	5.5	6	6.0	5	5.7	5.5
C	6.0	9	5.5	6	5.7	7.5
D	6.0	5	6.0	7	6.0	6.0
E	9.5	7	10.0	11	9.7	9.0
Total	33.0	33	33.5	32	33.1	32.5

TABLE IX.

Number of Gyres and Changes in Direction in Anaphase Chromosomes from Materials (3a) and (3b).

Chromosome	Asynaptic - Type (1)						Asynaptic -Type 2	
	Miscellaneous Chromosomes				Mean		Complete Nucleus	
	1		2					
	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes
A	11.0	4	-	-	11.0	4	17.5	4
B	15.0	0	14.0	4	14.5	2	20.0	9
C	12.0	8	10.5	4	11.2	6	16.0	3
D	14.0	6	15.0	4	14.5	5	21.0	12
E	20.0	8	-	-	20.0	8	30.0	14
Total	72.0	26			71.2	25	104.5	42

TABLE X.

Number of Gyres and Changes in Direction in Anaphase Chromosomes from Material (4) - Slide 58-2E-1.

Chromosome	Complete Nuclei								Miscellaneous Chromosomes						Mean	
	1		2		3		4		5		6		7			
	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes
A.	5.5	7	6.0	3	6.0	6	6.0	6	5.5	4	5.5	4	-	-	5.7	5.0
B.	6.5	4	7.5	8	7.0	4	7.0	6	7.0	5	6.0	6			7.0	5.5
C.	5.0	3	5.5	5	5.0	3	5.0	4	6.0	6	6.0	7	5.5	9	5.4	5.3
D.	7.5	7	7.0	8	7.5	7	7.0	5	-	-	-	-	-	-	7.7	6.8
E.	10.5	8	9.0	10	10.0	12	8.5	11	11.0	10	-	-	-	-	9.8	10.2
Total	35.0	29	35.0	34	35.5	32	33.5	32							35.6	32.8

TABLE XI.

Number of Gryes and Changes in Direction in Anaphase Chromosomes from Material (5) - Slide 58-4E-1.

Chromosomes	Complete Nucleus.		Miscellaneous Chromosomes										Mean	
	1		2		3		4		5		6			
	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes
A.	6	9	7.0	5	7	3	7.0	5	7	7	7	3	6.8	37.4
B.	8	6	7.5	10	7	4	8.5	11	-	-	-	-	7.7	7.7
C.	7	6	6.0	6	-	-	-	-	-	-	-	-	6.5	6.0
D.	8	8	9.0	10	8	5	-	-	-	-	-	-	8.7	7.7
E.	11	11	11.5	8	12	13	-	-	-	-	-	-	11.5	10.7
Total	40	40	41.0	39									41.2	37.4

TABLE XII.

Number of Gyres and Changes in Direction in Anaphase Chromosomes from Material (6). Slides 58-5H-3 and 58-5d-3.

Chromosomes	Anaphase I 58-5H-3		Anaphase I - 58-5d-3										Anaphase II 58-5d-3	
	Miscellaneous Chromosomes		Complete Nucleus	Miscellaneous Chromosomes									Complete Nucleus.	
			1		2		3		4		Mean			
	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes
A.	5.0	6	7.0	7	7.0	6	8	7	8	7	7.5	6.8	7	5
B.	8.0	10	10.0	10	9.5	12	9	12	-	-	9.5	11.3	8	7
C.	6.0	6	7.0	4	6.0	7	-	-	-	-	6.5	5.5	7	8
D.	8.0	8	9.0	9	8.5	6	-	-	-	-	8.7	7.5	8	6
E.	8.5	8	13.5	19	12.5	16	14	9	-	-	13.5	14.7	12	11
Total	35.5	38	46.5	49	43.5	47					45.7	45.8	42	37

TABLE XIII.

Number of Gyres and Changes in Direction in Anaphase Chromosomes from Material (7) Slide 65-L2-a.

Chromosome	Complete Nuclei				Mean	
	1		2			
	No. Gyres	No. Changes	No. Gyres	No. Changes	No. Gyres	No. Changes
A	9.5	3	10.0	4	9.7	3.5
B	11.5	9	12.5	11	12.0	10.0
C	9.0	3	9.0	7	9.0	5.0
D	12.0	10	11.0	13	11.5	11.5
E	16.0	16	18.0	16	17.0	16.0
Total	58.0	41	60.5	51	59.2	46.0

(1). It will be noted that, in a number of cases, two chiasma frequencies are given for the same material, one of which has been called the "effective" and the other the "true" frequency. In some cases these values differ quite markedly particularly where the chiasma frequency is high. The "effective" frequencies have been obtained by counting without the aid of drawings, while the "true" frequencies have been obtained by Mr. H.B. Newcombe from very careful analyses of camera lucida drawings. By the former method chiasmata which occur very close together are frequently counted as one while this error is not made in obtaining values from drawings. As will become clear later, two chiasmata or a chiasma and the attachment occurring within 1 or 2μ of each other would act as a single unit in their effect on coiling. The frequency which must be considered in relation to the effect of chiasmata on the direction of coiling would therefore be the "true" frequency minus such chiasmata which is called the "effective" frequency. That this value is practically the same as the value obtained by direct observation is indicated by the fact that these two figures agreed almost exactly in the case of slide 58-5d-3 where the values obtained in both ways were the result of a sufficient number of observations to make the comparisons reliable. When the chiasma frequency is relatively low, as in slide 65-L2-a, "effective" and "true" frequencies are practically the same.

(2). In Huskins and Smith's and Dr. Hunter's material the figure given by the former authors is considered to be the

TABLE XIV.

Chiasma Frequencies of Materials Analysed for Changes in Direction of Coiling.

	Material	True Xa frequency	No. cells	Effective Xa frequency	No. cells
1.	From Huskins and Smith (1935) and Dr. Hunter	21.8 ± 0.84	6	17.2 ± 0.47	20
*2.	Desynaptic - Slide NW-4L-1-15	-	-	10 - 17	-
3.	Asynaptic Type (1)	0.0	0	0.0	0
	Asynaptic Type (2)	0.0	0	0.0	0
4.	From Slide 58-2E-1	-	-	14.4 ± 1.87	8
5.	From Slide 58-4E-1	19.0	2	17.6 ± 1.27	10
6.	From Slide 58-5H-3	-	-	20.0 ± 1.68	12
	From Slide 58-5d-3	21.3 ± 2.42	12	17.5 ± 2.09	12
7.	From Slide 65-L2-a	14.1 ± 2.76	12	13.3 ± 1.41	12

* Chiasma frequency estimated.

effective chiasma frequency while the figure determined by Mr. Newcombe in two plants of the same material by tracing threads through chiasmata in camera lucida drawings is considered to be the "true" frequency.

(3). The chiasma frequency of the desynaptic material could not be determined since all stages observed were too late. A few cells, however, showed bivalents with one or two chiasmata and one cell had a frequency of nine all of which were on the point of being resolved. From these observations it was believed that the lowest possible limit would be about five and it seemed probable that it may be higher. Arbitrary limits of 10 and 17 were, therefore, chosen for purposes of analysis.

4. Relational Coiling.

As mentioned above, the chromatids of the first pollen-grain division chromosomes of Trillium are found to be relationally coiled. Indications of this coiling may be seen as soon as the doubleness of the chromosomes becomes apparent in early prophase. From mid-prophase to metaphase it is possible to analyze these twists with a considerable degree of accuracy.

The observations which have been made during the present investigation must be considered to be preliminary to a more elaborate study of this problem. Such data as have been obtained, however, seem worth presenting since they do establish several

facts and give a number of clues which may prove valuable in further work.

The data presented in Tables XV-XIX have been obtained from materials which passed through meiosis under the following conditions:

(1). At 15°C in a temperature chamber during the winter of 1936-37 (Corm NW-5E-2).

(2). At 15°-23°C in the laboratory during the winter of 1938-39 (Corms 65-M and 65-P).

(3). At about 3°C in the refrigerator until first to second anaphase after which the corms were transferred to the laboratory at 18°-22°C (Series 63-RC). This material was also from the 1938 collection.

TABLE XV.

Number and Direction of Relational Twists at Mid-Prophase of Pollen-Grain Division - Corm 58-5E-2.

	No. of twists	Direction of twists		No. of twists	Direction of twists
1.	2	RL	9.	3	RRR
2.	4	RRRR	10.	4	LLLL
3.	0	-	11.	0	-
4.	0	-	12.	3	LLR
5.	4	RRRR	13.	2	RR
6.	3	LRR	14.	3	LLR
7.	5	LLLLL	15.	1	R
8.	4	LLLL			
			Total	38	19R 19L

TABLE XVI.

Number and Direction of Relational Twists at Metaphase of Pollen-Grain Division - Corm NW-5E-2.

Chromosome:	A		B		C		D		E		Total
Cells	No. twists	Direct-ion of twists	No. twists	Direct-ion of twists	No. twists	Direct-ion of twists	No. twists	Direct-ion of twists	No. twists	Direct-ion of twists	No. twists
1.	1	-.L	0	U.U	0	U.U	1	U.R	2	R.R	4
2.	1	-.L	2	R.R	1	U.R	0	U.U	3	L.LL	7
3.	0	-.U	0	U.U	0	U.U	0	U.U	0	U.U	0
4.	1	-.R	3	U.RLL	1	U.R	0	U.U	2	RL.U	7
5.	0	-.U	1	U.L	0	U.U	1	R.U	1	U.L	3
6.	0	-.U	1	U.L	1	U.L	0	U.U	2	R.L	4
Total	3		7		3		2		10		25
Mean	0.5		1.2		0.5		0.3		1.7		4.2

The Direction of Relational Twisting on Either Side of the Attachment.

Direction	R.R	R.L or L.R	L.L	R.U	L.U	U.U
Number	2	1	1	5	5	10

TABLE XVII.

Chromosome Lengths During First Pollen-Grain Division - Corm NW-5E-2.

Prophase.

Cell	Chr.: A	B	C	D	E	Total
1.	34	43	30.0	46.0	62.0	214.0
2.	31	35	29.5	40.5	62.0	198.0
3.	34	42.5	30.0	44.0	46.0	197.0
Mean	33	40.2	29.8	43.5	56.7	203.2

Metaphase.

Cell	Chr.: A	B	C	D	E	Total
1.	18.5	13.5	12.3	17.8	27.6	89.7
2.	14.8	14.1	12.9	17.7	24.0	83.5
3.	16.6	20.1	15.4	16.0	22.0	90.1
4.	16.6	16.5	16.0	19.7	28.8	97.6
5.	16.0	15.4	14.8	17.8	24.0	88.0
6.	11.0	14.0	11.5	12.0	18.0	66.5
7.	7.0	10.0	8.0	11.0	20.0	56.0
Mean	14.3	14.8	12.9	16.0	23.5	81.5

TABLE XVIII.

Chromosome Lengths and Number and Direction of Relational Twists During First Pollen-Grain Division Series 65-P and 65-M.

Chromosome:	A			B			C			D			E			Total	
Cell	Chr. Length	No. twists	Direction of twists	Chr. Length	No. twists	Direction of twists	Chr. Length	No. twists	Direction of twists	Chr. Length	No. twists	Direction of twists	Chr. Length	No. twists	Direction of twists	Chr. Length	No. twists
1.	26	2	-.RL	31	2	R.R.	27	2	LL.U	33	6	RR.LRLR	51	5	L.LLLR	168	17
2.	21	2	-.LL	27	2	U.RL	21	3	LL.L	27	2	R.R	27	2	L.L	123	11
3.	23	6	-.RRRRRR	20	2	U.LL	20	1	R.U	21	1	U.R	34	3	U.LRL	118	13
4.	20	2	-.LL	16	3	U.RLR	17	3	RR.R	22	4	RR.RL	34	6	LLL.LRR	109	18
5.	13	3	-.RRL	18	1	R.U.	14	1	R.U.	16	1	R.U	24	3	LL.L	85	9
6.	15.5	2	-.LL	15	2	U.RL	14	4	U.LLLL	18	3	U.RRR	20.5	3	L.RR	83	14
7.	13	2	-.RL	14	2	U.RR	12.5	1	R.U	14.5	1	R.U	21	3	RR.R	75	9
8.	12.2	1	-.L	15	1	U.R	9.2	0	U.U	16.2	0	U.U	19.4	1	U.L	72	3
9.	11	4	-.LRRR	12	1	R.U	11	2	U.LL	15	0	U.U	23	4	RR.RR	72	11
10.	13.5	2	-RL	14	1	U.R	10	2	U.LL	16	2	R.R	17	2	R.L	70.5	9

The Direction of Relational Twisting on Either Side of the Attachment.

Direction	R.R	R.L or L.R	L.L	R.U	L.U	U.U
Number	7	3	5	14	5	3

TABLE XIX.

Chromosome Lengths and Number and Direction of Relational Twists During First Pollen-Grain Division Series 63-RC.

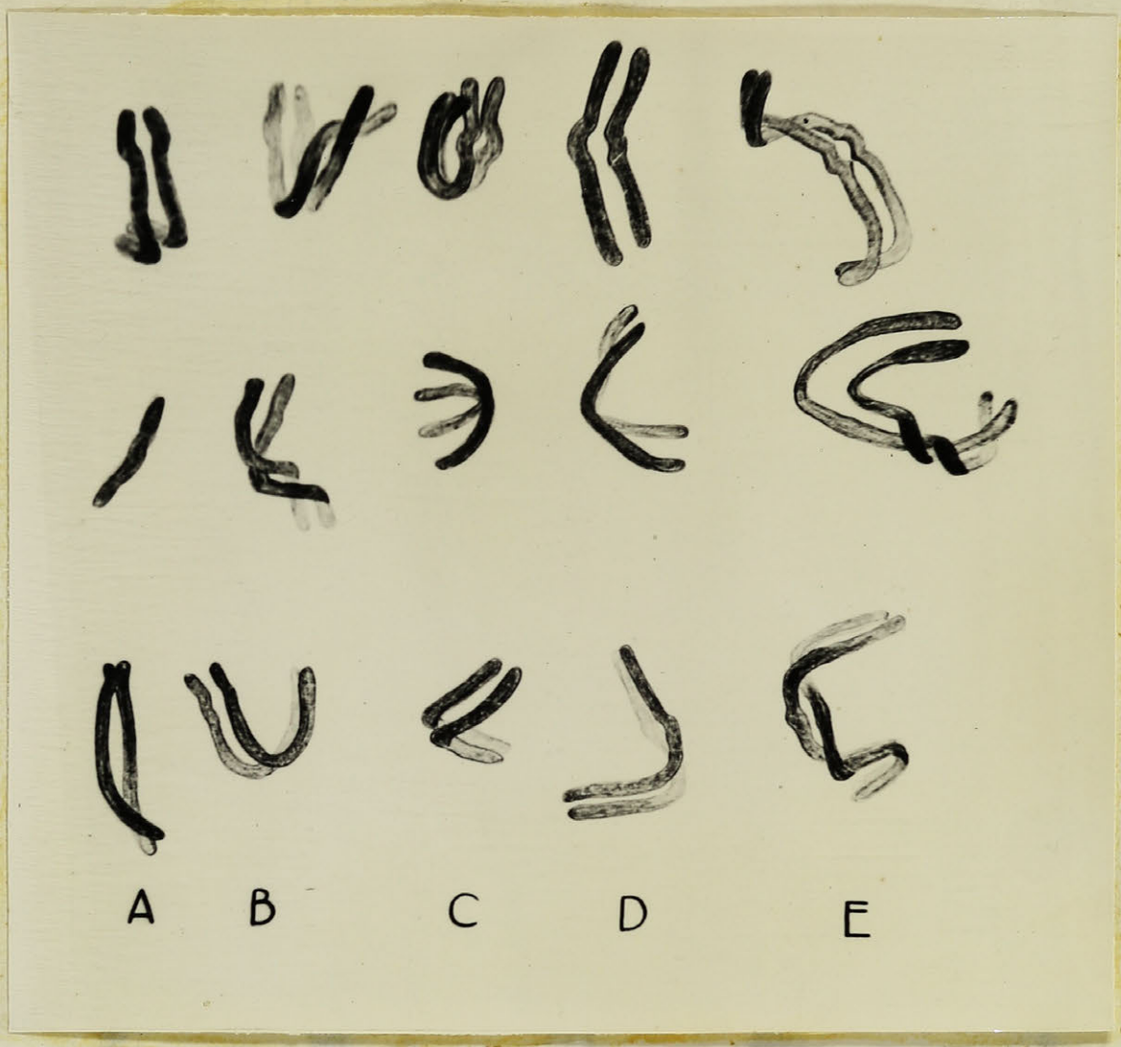
Chromosome:	A			B			C			D			E			Total	
Cell	Chr.Length	No.Twists	Direction of twists	Chr.Length	No.Twists	Direction of twists	Chr.Length	No.Twists	Direction of twists	Chr.Length	No.Twists	Direction of twists	Chr.Length	No.Twists	Direction of twists	Chr.Length	No.Twists
1.	20	2	-.RR	25	5	U.RRLRL	20	3	L.LL	29	3	R.RR	40	4	LR.RR	134	17
2.	25	2	-.LL	22	1	U.L	18	0	U.U	24	1	U.L	31	1	U.L	120	15
3.	23	1	-.R	18	2	U.RR	18	5	LL.LLL	23	1	R.U	36	3	LR.L	118	12
4.	21	1	-.L	18	0	U.U	16	2	R.R	22	3	RR.L	30	2	L.R	107	8
5.	20	4	-.RRRR	22	1	U.L	17	2	L.R	19	1	U.L	24	1	U.L	102	9
6.	16	2	-.RL	19	1	L.U	17	1	U.R	19	4	LL.RR	30	2	U.RL	101	10
7.	21	0	-.U	19	0	U.U	12	2	R.L	17	2	R.L	27	2	R.L	96	6
8.	19	1	-.R	18	2	U.RR	17	1	R.U	19	2	U.RR	22	4	R.LRR	95	10
9.	14	2	-.LL	18	1	U.R	14	0	U.U	16	2	U.LL	24	2	U.RL	86	7
10.	11	1	- LL	18	2	U.RR	13	1	U.L	19	0	U.U	25	3	R.LL	86	7
11.	13.5	2	- RR	16	1	U.R	10	2	L.R	17	1	U.R	25	1	U.R	81.5	7

The Direction of Relational Twisting on Either Side of the Attachment.

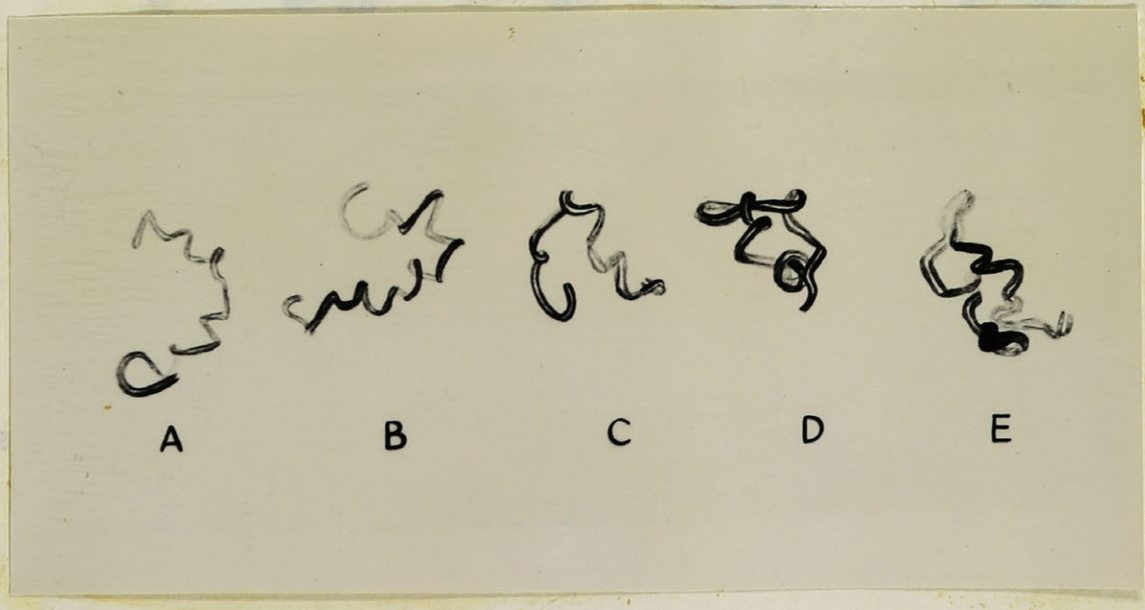
Direction	R.R	R.L or L.R	L.L	R.U	L.U	U.U
Number	3	11	2	14	9	5

The first of these materials was analyzed by Mr. MacKenzie, whose results are given in Tables XV, XVI, and XVII.. The number and direction of twists were determined for six complete cells at metaphase and for fifteen miscellaneous mid-prophase chromosomes. In the latter, it was, for the most part, impossible to identify the chromosomes, and no complete cell was found which could be accurately analyzed at this early stage. Unfortunately Mr. MacKenzie did not draw all his figures with the camera lucida or make micrometer measurements in every case. This omission renders it impossible to compare the number of twists with the corresponding chromosome length. He did, however, measure the chromosomes in seven metaphase cells which were chosen because of the ease with which accurate measurements could be made and three of these cells (Text fig. 15) were included in his analysis of relational coiling. Measurements were also obtained for three prophase cells (Text fig. 16) which were slightly earlier than those analyzed for relational twists, but in which it was possible to identify the chromosomes quite accurately. It is therefore possible from these data to make fairly reliable comparisons between the number of twists and chromosome lengths both at mid-prophase and metaphase.

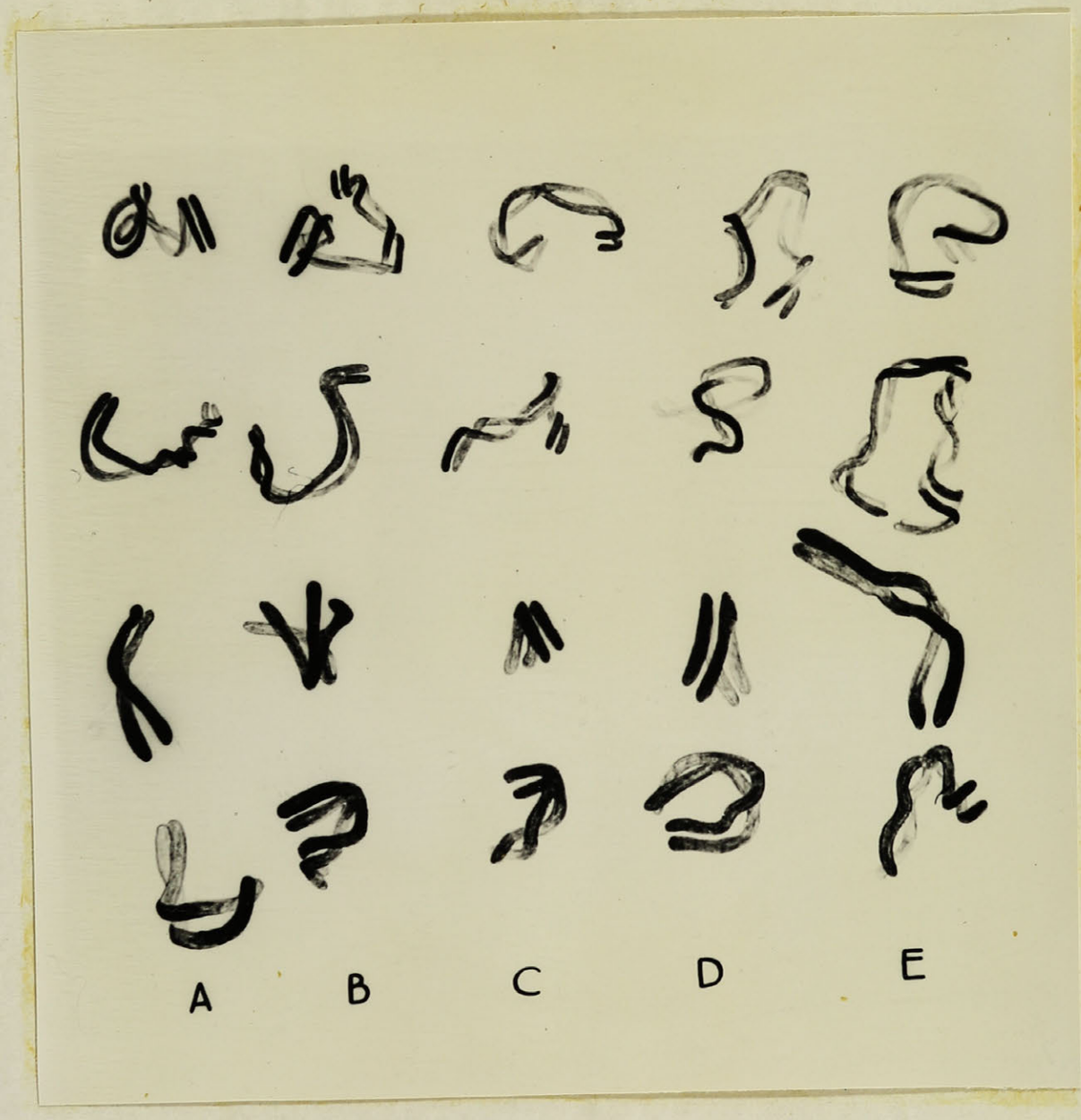
All drawings of the remaining two materials (see Text figs. 17, 18 as illustrations) were made by means of the camera lucida and measurements are, therefore, available. It was found impossible to analyze stages as early as the mid-prophase



Text fig. 15. The chromosome complements of three cells at metaphase of first pollen-grain division in material NW-5E-2.



Text fig. 16. A chromosome complement from prophase of first pollen-grain division. Note the relic coils.



Text fig. 17. The chromosome complements of four cells from first pollen-grain prophase and metaphase in material 65-P.

Text fig. 18. The chromosome complements of four cells from first pollen-grain prophase and metaphase in material 63-RC. Note the attachment regions in the chromosomes of the third row.

chromosomes of the first material, late prophase being the earliest stage which could be interpreted with sufficient certainty. Tables XVIII-XIX give the chromosome lengths and the number and direction of the twists for all the cells analyzed.

DISCUSSION.

Most of the data which have been presented are more or less directly concerned with the question of coiling. In this section an analysis of these will be undertaken in order to determine, in so far as possible, the nature of their relationship to coiling. Beyond reporting observations, factors which are not obviously associated with coiling will not be discussed further. The general appearance and behaviour of the chromosomes during division, the physical characteristics of the attachment region and, excepting the stage at which coiling occurs, the number of threads in the chromosome belong in this category.

With the possible exception of the protista, it is probable that the chromosomes of all organisms exhibit coiling at some stage of the division cycle. While the details may differ in different cases it seems reasonable to expect that the underlying principles are always the same. If this is so, an analysis of a specific case should give some information of general utility.

Numerous studies have shown that two types of coiling occur, a spiral or helical coil and a relational twisting like that in the two strands of ordinary electric-flex. Both types have conclusively been shown to be characteristic of the chromosomes of a wide variety of organisms. The spiral type

is most clearly seen at first anaphase of meiosis while the relational twisting is especially characteristic of the chromatids of late prophase and early metaphase in mitotic divisions. In the present case the former type is represented by the major or meiotic coil and the latter by the relational twisting of the prophase and metaphase chromatids of the first pollen-grain division in Trillium erectum. The major spiral will be considered first.

The Major Coil.

As a sample coil this spiral has certain advantages. During its development the chromosomes are large and can be easily distinguished from each other, internal structure may be disclosed with comparative ease and all four threads of a bivalent may be studied individually since sister strands are not as closely associated as is the case in Tradescantia.

As pointed out by Huskins and Smith (1935) this coil is in the process of formation from diakinesis to early anaphase. Great care has been exercised during the present study to check this statement with the result that absolutely no evidence could be found for any other view. It is, therefore, considered to be a fact that the coil is initiated as a series of more or less regular loops which gradually becomes a three dimensional coil. This, then, establishes two points of significance: (1) there is a time element

involved in coiling and (2) coiling is progressive from a relatively straight thread to a spiralled one.

With these points established, the next logical step is to determine what changes, if any, occur in the chromonema or chromosome, or both, during the period of coiling. An obvious change to be expected is one of length, and measurements were, therefore, made at diakinesis and first anaphase on both chromosomes and chromonemata. The results of these have already been given (cf. Tables II-VI). In making these measurements materials from five sources were used, as previously described, and, although the numbers within each group are quite small, the total number of chromosomes is fairly large. In each case the ratio of anaphase to diakinesis lengths is approximately 2 which means that the chromonemata have doubled their length between the two stages and during coiling. If, instead of taking the groups individually, we consider all the data together, there are 114 diakinesis lengths to compare with 76 anaphase measurements. We note then that when any chromosome of the set at diakinesis is compared to the corresponding one at anaphase, the ratio is still in the same direction; e.g., the chromonema length of the A chromosome at diakinesis in any group is shorter than any A chromosome at anaphase.

Great as these differences are, it would be unwise to accept them without first examining the methods of measurement for sources of error in order to determine, if possible, whether

or not such errors would be great enough to consume the difference. As described earlier, chromosome measurements were made both by the use of a micrometer eye-piece, calibrated for the objective used, and camera lucida drawings. The latter method was also used in measuring the chromonemata at diakinesis. Anaphase chromonema lengths were calculated in two ways: (1) with the one-plane measurement taken from the drawings as a basis and (2) by applying the coil formula.

These methods have already been described in detail and the chief sources of error indicated. It is possible, of course, that the errors which cannot be excluded by the checks used are serious enough to render the conclusions invalid. When, however, all the chromonema measurements are considered together regardless of the groups, the difference between the mean lengths at diakinesis and anaphase is about five times the probable error and therefore quite significant.

From this data, then, a third point can be established: <u>The chromonema increases during coiling to about twice its early diakinesis length.</u>

Another point of interest lies in whether or not the end-to-end or chromosome length alters during the coiling period. It is clear from the data as recorded in the tables that any difference which may exist between diakinesis and anaphase chromosomes in this respect is very slight since

it rarely amounts to more than 4-5μ in 50-70μ. Such a difference, particularly in view of the relatively crude methods of measurement used, might well be expected to be within the margin of error. It is of interest to note, however, that the ratio of anaphase to diakinesis mean lengths for each of the materials, excluding, of course, the uncoiled chromosomes from slide 58-6d-3, is consistently less than unity (Table XX). If individual nuclei at anaphase are compared with those of the same material at diakinesis, this does not invariably hold. In this case only about 75% of the ratios are less than one. This probably means that the mean chromosome length at anaphase is less than the mean length at diakinesis but that the individual variations are greater than this difference. Some significance may also be attached to the fact that the methods by which the diakinesis chromosome lengths were measured did not take into account the looping out between chiasmata. In many cases this must have given rise to a fairly substantial error which, if taken into account, would increase the diakinesis lengths. From these considerations a fourth point would seem to be indicated: <u>a very slight contraction of the chromosome takes place during coiling.</u>

These two phenomena of chromonema elongation and chromosome contraction occur at sometime during the period in which the major coil is being developed. There is, however, no very strong evidence from the figures themselves

TABLE XX.

The Ratios of Anaphase to Diakinesis Chromosome and Chromonema Lengths.

Data from Table	Mean Chr. Length		Ratios	Mean Ch'ma Length		Ratios
	Ana-phase	Diaki-nesis		Ana-phase	Diaki-nesis	
II	51.9	52.4	0.97	202.4	84.4	2.42
III	51.9	54.3	0.96	201.0	67.6	2.97
IV Ana.I	64.6	66.4	0.97	211.0	83.2	2.54
IV Ana.II	53.3		0.81	183.1		2.21
V	69.5	77.8	0.89	219.6	105.9	2.07
VI	124.4	94.8	1.32	131.1	103.0	1.28

TABLE XXI.

The Variablity of Chromosome and Chromonema Lengths During Diakinesis and Anaphase*.

Stage	Mean Chr.Length	σ	Coeff. of var.	Ch'ma Length	σ	Coeff. of var
Diak.	65.1	9.9	15.2±2.18	82.5	19.8	24±3.6
Ana	58.5	8.6	14.7±3.1	201.7	16.4	8±1.6

*Figures calculated from cell totals in Tables II-V.

to show that elongation and contraction are also gradual processes. There is a possibility that they may be abrupt changes. If, however, the variability of the chromonema lengths were found to be greater at diakinesis than at anaphase it would constitute rather strong evidence of the gradual nature of the elongation process. To determine whether or not this is so the coefficients of variability were calculated for the chromonema and chromosome lengths at the two stages. The data used for these calculations were taken from all complete nuclei given in Tables II-V. As shown in Table XXI the diakinesis chromonema lengths are the more variable while the chromosome lengths show practically the same degree of variability at both stages. Since this difference in variability between the chromonema lengths at the two stages is statistically significant while that between the chromosome lengths is not it would seem to indicate that chromonema elongation is a gradual process occurring during diakinesis while chromosome contraction is more abrupt. The degree of chromosome contraction is so slight, however, that even if it is gradual this method would scarcely be expected to show it.

From these considerations it is quite probable that chromosome and chromonema length changes are associated with coiling. Before a casual relationship can be established, however, it is necessary to demonstrate some more concrete link between them than that of time alone.

Most techniques of fixation and staining cause the chromosomes to appear as solid rods devoid of an internal structure. It is generally, but not universally, considered that this body represents a matrix within which the chromonemata lie. While this is an obvious and reasonable assumption, it is not necessarily correct on the basis of this evidence alone. The apparent matrix may, for instance, be a vital artefact or, more concretely, it might be imagined that the special methods used to show internal structure shrink the chromonema which is ordinarily a thick rod spirally wound and occupying the whole of the stained area. This latter, however, does not seem to be a very satisfactory hypothesis. A thread as thick and as short as an early diakinesis chromosome would not give the number of anaphase coils observed, and, to argue the other way, if the solid-appearing rod at anaphase is made up of gyres so closely wound that there are no inter-gyral spaces then the diakinesis chromosome would be much longer and thinner than it is.

It may also be pointed out that when the chromosomes are stained for structure, there remains a hyaline area about the chromonemata which is the same shape and size as the mass stained chromosome would be. Of course this may only mark the region from which the chromonemata have shrunk under the action of the fixative. In many aceto-carmine preparations, however, both the chromosome and chromonemata are visible as

stained structures at the same time. Dr. Hunter's asynaptic chromosomes are excellent examples of this. Furthermore, unstained and unfixed cells mounted in 4% cane sugar show dark coils lying within a lighter region which is in turn differentiated from the surrounding cytoplasm. While none of these points need be considered as proving the presence of the matrix, they do make an assumption of such a structure more logical than the contrary opinion. The most critical evidence on this point is, I believe, supplied by the material on slide 58-6d-3. There is no sign of a hyaline area about either diakinesis or anaphase chromosomes in this material. It is, therefore, something which may or may not be present, in short, a physical entity. It is also noteworthy that its absence is accompanied by the absence of coil formation but not of elongation. For these reasons the matrix is here considered to be a real structure and to its outside surface the term pellicle or sheath has been applied. It is this pellicle which provides the necessary physical link between elongation and coiling. Therefore, a fifth point may justifiably be added: the boundary of the chromosome has, at certain stages, the properties of a sheath within which the chromonema is imprisoned.

With these five points in mind, coiling may now be examined from a mechanical point of view. If these points can be considered as established, then the following statement may be made: the chromonema lies within a pellicle which,

if it changes at all, contracts slightly while the chromonema is elongating. The expected result of these conditions is a spiral. As far as the present data on Trillium are concerned this is a logical explanation of coiling provided that the chromonema has some elasticity. Since it is essentially protein in nature a certain degree of elasticity may be taken for granted; specific evidence of which is available from many studies of which that provided by Chambersand Sands (1923) from the micro-dissection of Tradescantia chromosomes seems conclusive.

Whether or not the chromonema has an internal molecular arrangement which facilitates coiling cannot, of course, be determined directly. The assumption that such an arrangement exists is the basis for all torsion theories of coiling and Darlington (1935, 1937) in particular has used this idea as the basis of a general hypothesis of coiling. While the hypothesis is quite logical in itself, certain observations provide serious difficulties. Changes in direction of the major spiral (cited herein) and of the relational twists (Sax, 1936) cannot be explained satisfactorily. Darlington himself (1937 p.489) has recognized the difficulty of explaining the direction of relational coiling in certain cases of chiasma formation.

The coincidence of certain events with a particular phenomenon is, of course, not considered to be a proof of

causal relationship. If, in the present case, the completed coil should show some property or properties not explicable in a spiral resulting from the proposed mechanism of coiling, it would be necessary to consider the hypothesis invalid. It is, therefore, an integral part of a study on coiling to determine also the properties of the completed coil. In this connection three questions seem to be of considerable importance: (1) Is the direction of coiling constant throughout the length of a chromonema or not? (2) What relation as to the direction of coiling exists between sister strands and between homologous strands? (3) How and when does uncoiling take place? The answers to these questions, as obtained from the present observations, may disprove the hypothesis or strengthen it. It must be remembered, however, that a hypothesis cannot be proved but can only be made tenable or untenable.

To the first question the answer may be made directly. The direction of coiling may change at the attachment and interstitially. Such changes have been found in synaptic material by Huskins and Smith, Dr. Hunter and during the present study. Similar changes also occur in asynaptic material (Dr. Hunter). The direction of coiling is, therefore, not consistent within the chromonema.

In regard to the second question Dr. Hunter's two types of asynaptic material answer this quite adequately. In the first type sister chromatids are closely associated and

always coil in the same direction while in the second type sister strands are unassociated and coil at random with respect to each other. These two types obviously constitute the extremes in association and, therefore, synaptic material should be intermediate. If four strands are random in their direction of coiling, 50% of the corresponding gyres should show three coiling in one direction and one in the other. All the anaphase chromosomes illustrated by Huskins and Smith and Dr. Hunter (three A, five B, three C, four D and four E bivalent chromosomes) were analyzed in this way with the result that 38% of the cases showed this three and one condition. Two cells picked at random from the present material were also analyzed and showed approximately 25% of the three and one condition. It is, therefore, clear that the synaptic material is intermediate between the two types of asynaptic material.

It is, of course, evident from this that randomness in the direction of coiling is inversely related to the closeness of association.

The third question cannot be answered with as great assurance. There does, however, seem to be adequate reasons for assuming that the relic coil of first pollen-grain division prophase is that of the major coil. If so, then unravelling occurs during prophase and is concurrent, in part at least, with contraction. As to how uncoiling occurs it may be said that it appears to follow the uncoiling procedure expected of

any more or less elastic thread wound into a coil. As soon as the force responsible for the preservation of the coil is removed unravelling would take place and the speed with which this occurs would depend on the viscosity of the medium in which it is occurring. In the hypothesis of coiling proposed above, the force responsible for the preservation of the coiled state is the pellicle. It has already been pointed out that this is disappearing at second telophase and is presumably entirely eliminated by the time resting stage is reached. This poses a question which may be answered only by making a number of assumptions, evidence for which is altogether too slight. Since the resting stage prior to pollen-grain division is long compared to prophase it may be asked why uncoiling does not occur then. That it does not, to more than a very slight extent at least, has already been indicated in that the earliest prophase chromosomes are still found to be coiled almost as tightly as in meiosis. At the same time, there seems every reason to believe that the coil is without a restraining pellicle during resting stage. A possible explanation, however, may be evolved from the following considerations: (1) the viscosity of the medium in which the chromosomes are uncoiling at prophase of mitosis is great enough to cause a very noticeable lag in uncoiling, (2) such viscosity cannot be characteristic of the prophase of meiosis since there is little or no sign of relic coils at leptotene, (3) According to Beasley (1938) the meiotic prophase nucleus is larger

than the mitotic prophase nucleus and probably the latter is the more viscous . If such a relation exists between size and viscosity then the resting nucleus would be the most viscous of all and may take the place of the pellicle as a force for preserving the coiled state of the chromatid.

The properties of the completed coil are such as would be expected of a spiral formed by the mechanism proposed. Changes in direction should occur at random at any point of interruption, two threads coiling together within the same pellicle should coil in the same direction while those which are unassociated should coil in random directions with respect to each other, and uncoiling should occur in the way described. This last statement, of course, would apply to any coil of more or less elastic material.

It has not, of course, been shown that changes in direction occur at points of interruption. If this can be shown and if it can also be established that they are probably random at such points then the hypothesis that coiling is due to differential length changes between the pellicle and its enclosed chromonemata becomes established as a satisfactory explanation for the specific case of the major coil of Trillium erectum.

In a normally synaptic bivalent there are three possible "interrupting" factors: (1) the attachment; (2) chiasmata; and (3) the longitudinal heterogeneity of the chromonema which

should have an effect proportional to length. In asynaptic material, of course, chiasmata are not present and all changes should be associated with the remaining two factors.

The number of changes which would be expected to be associated with the attachments and chiasmata can be directly derived from the number of attachments and the chiasma frequency. If changes are random at both these points, the number of changes at the former should equal half the attachments per cell while the number of changes at the latter would be equal to the chiasma frequency since each chiasma involves two threads. Any changes which were not found to be due to these two factors must be due to the third one and would be proportional to the number of gyres, and the ratio of changes to number of gyres should be the same for all material. In Table XXII the number of changes per cell have been divided into the three possible classes and the ratios of the total number of changes to the number of gyres and the total number minus those assumed to be associated with the attachments and chiasmata to the number of gyres have been calculated.

Any analysis of this type is, of course, subject to limitations. Certain explanations must, therefore, be given before an attempt is made to derive a meaning from this table. Some of these explanations have already been considered earlier when the data on changes in direction and chiasma

TABLE XXII.

The Ratios of the Number of Changes in Direction per Cell to the Number of Gyres per Five Chromatids.

	Material	No. Changes	No. Gyres	No. Changes / No. Gyres	True Xa frequency	Effective Xa frequency	No. changes assoc. with xta	No. changes assoc. with attachment	Remaining no. changes	Remaining Changes / No. Gyres
*1.	Synaptic	42.6	57.8	0.74	21.8	17.15	17	8	17.6	0.30
2.	Desynaptic	32.5	33.1	0.95	-	10-17	10-17	8	14.5-7.5	0.44-0.23
3.	Type (1) asynaptic	25.0	71.0	0.36	0	0	0	8	17.0	0.24
	Type (2) asynaptic	42.0	104.5	0.40	0	0	0	8	34.0	0.33
4.	Slide 58-2E-1	32.8	35.6	0.90	-	14.4	14	8	10.8	0.30
5.	Slide 58-4E-1	37.4	41.2	0.91	19.0	17.6	17	8	12.4	0.30
	Slide 58-5H-3	38.0	35.5	1.08	-	20.0	20	8	10.0	0.28
6.	Slide 58-5d-3 Ana.I	45.8	45.7	1.00	21.3	17.5	17	8	20.8	0.45
	Slide 58-5d-3 Ana.II	40.0	42.0	0.95			17	8	15.0	0.37
7.	Slide 65-L2-a	46.0	59.2	0.78	14.1	13.3	13	8	25.0	0.42

*From Huskins and Smith (1935) and Dr. Hunter.

frequencies were set forth. It is, however, convenient to have an analysis of the table prefaced by an explanation of all points which are not self-explanatory. For this reason the following points may be considered:

(1). The number of changes and gyres are the means from Tables VII to XIII.

(2). The "effective" chiasma frequency was obtained by direct observation without camera lucida drawings while the "true" frequency was obtained by careful analysis of drawings. The latter frequencies include a number of chiasmata which are within 2μ of each other or within a similar distance of the attachement. Since on an average, a gyre comprises over 2μ of the chromonema it is quite obvious that the "true" chiasma frequency is too high for use in the analysis here presented because two chiasmata or a chiasma and the attachment, if close together, will function as one point of interruption. Unless drawings are made, chiasmata which are very close together are generally counted as one; thus the frequencies obtained without drawing are lower than the true value. Since the objective of the present analysis is to determine the effect of chiasmata on the direction of coiling the lower values are clearly the ones to be considered.

(3). The chiasma frequency for the desynaptic material could not be determined but was estimated to be between 10 and 17 at the time of coiling. This estimation

was based on the number of terminal chiasmata still present after coiling was completed and on the frequency of chiasmata in more normal material under similar conditions.

(4). Changes in direction are random at the attachment and there are twenty attachments in an anaphase cell. Four of these, however, are the terminal ones of the A chromosome and would not affect the direction of coiling. Thus the number of changes per cell associated with the attachments should be about eight.

With a few exceptions, the ratios of the number of changes left after subtracting those assumed to be associated with the attachements and chiasmata to the number of gyres is remarkably constant for all materials while the ratios of the total number of changes to the number of gyres are, for the most part, quite variable. The coefficients of variability have been determined for the two series of ratios and have been found to be 1.2 for the former and 6.6 for the latter. This must mean then, that part of the changes are associated with the number of gyres while the rest depend on the attachments and chiasmata.

Apparent serious exceptions are found in first anaphase chromosomes from slides 58-5d-3 and 65-L2-a since the ratios are considerably different from those of other materials. In both these cases, however, the number of changes could not be determined accurately since the chromo-

nemata were very loosely coiled and what may have been a non-coiled loop was probably often called a change in direction.

It will be noted that the ratio of the total number of changes to the number of gyres is practically the same in several materials, which might be taken to indicate that all changes are proportional to the number of gyres (cf. Matsuura, 1937). This similarity in the ratios would be expected, however, in materials which are similar in regard to number of gyres and chiasma frequencies or in which the ratios of these were similar. That this is so can readily be ascertained from the table. It may also be pointed out that if all changes were directly dependent on gyre number the ratios of the two asynaptic materials would be inexplicable.

It may, therefore, be concluded that changes in direction of the major coil are probably associated with three factors in the following way: the direction of coiling is (1) random on either side of the attachment, (b) random on either side of a chiasma and (c) varies directly with the number of gyres. This last factor is probably determined by the heterogeneity of the chromonema itself.

The Minor Spiral.

With Fujii's report in 1926 that in addition to the major coil of meiosis there was also present a small gyred coil running at right angles to the large spiral, a further

complexity was introduced into the subject of coiling. Since that time the minor spiral concept has been generally accepted in Japan by Kuwada and his school, and in England by Darlington and his co-workers. American workers are divided in opinion and on the whole tend to be more or less neutral on the subject. It is, of course, not surprising that there should be differences of opinion on a structure as near the limits of resolution as the minor spiral must be. It is illustrative of the lack of certainty among the adherents of the minor spiral concept that their interpretations are in keeping with their opinions on the number of threads present in the chromosome at the time. Twelve years of intensive study on the structure of chromosomes have failed to establish a final answer. It is, therefore, obviously difficult if not impossible to give a definite answer to the question of its existence. The small-gyred coil of somatic chromosomes or that produced by stretching or distortion of the major coil of meiosis must not of course be confused with the "minor" coil assumed by the Japanese workers to run at right angles along the length of the meiotic major coil.

During the present study as well as throughout other investigations on the structure of chromosomes made in this laboratory, evidence on the minor spiral has been sought, To date there has been no indication that such exists, but, of course, this is not enough to render the idea void even for Trillium much less for other plants. It has been noted, however,

that various optical illusions which could very easily be interpreted as minor spirals occur. In cases, for instance, where the tertiary split is not clear alternate dark and light bands are sometimes seen which could be interpreted as a spiral. However, where the split can be resolved the minor spiral disappears.

Perhaps little is to be gained by pointing out such examples but it does seem that the realization that such illusory parallels are occasionally seen may act as a caution sign. At such limits the eye becomes not only non-infallible but may even successfully trick the observer. A consideration of the more obvious cases of illusion may, therefore, serve as a means of tracking down the less obvious cases. The following have been selected for this purpose:

(1). In a pachytene of oats it was noticed that at certain places there was apparently a single thread wound into a small and fairly tight spiral. Further observation disclosed places in which the thread was bipartite and even faintly quadripartite but without a spiral. This spiral appearance is, therefore, quite certainly due to the merging of four threads of uneven surface into one apparent thread.

(2). Focusing on the turns of the spirals in two chromatids coiled closely together as is the case in associated sister strands, brings into view four transverse bars which may be "joined" to form a spiral running at right angles

to the major spiral. In this case the trick is obvious but such would not be the case were the two threads less distinct, as is most often the case with half-chromatids formed by the tertiary split.

(3). Two lines of dots running side by side and staggered if close enough together will appear to the eye as a zig-zag line or, if one imagines the third dimension, a spiral. Two such lines of chromomeres would, therefore, give the same impression and if they were not clear such an impression might be difficult to eradicate.

The conditi ons present in these three samples are probably also present in the chromosome at the time which is marked by the supposed presence of the minor coil. If these conditions are present they would result in an illusion and how are we, then, to distinguish between the real and the unreal?

Few drawings and even fewer photographs of the minor spiral have been published. By far the best photograph was given in Oura's paper in 1936. This stands today as the strongest evidence for the existence of a minor spiral. It is extremely unfortunate that the published figures seem to have been touched up so as to emphasize the point which the author was trying to make. Such an occurrence must needs have an unfavourable reaction. Any condemnation of the author on this point is, however, entirely unjustified. The original un-touched prints (which he kindly sent to Dr. Huskins

through Prof. Kuwada) bear out his words just as well as the published figures. It must be noted, however, that there are in the same cell apparent minor spirals of two magnitudes, the larger at the top around 12 o'clock and the smaller at the bottom around 6 o'clock. The former is obviously an optical illusion due to the juxtaposition of two chromatid spirals while the latter is in sister strands slightly out of step and is the real minor spiral if such exists at all. The minor spiral, then, becomes so minute that it is impossible to say whether or not it is more than a one-plane waviness. Add to this the fact that Kuwada and his school, like ourselves, are convinced that each chromatid is longitudinally double at this time and the impossibility of settling this problem by direct observation with the existing optical instruments becomes obvious. The one outstanding photograph of a minor spiral thus becomes at best no more than a positive indication.

In all the work on Trillium no clear cut indication of a minor spiral has been seen. The nearest to such a structure that has been observed is a slight waviness in the half-chromatide at first anaphase. Certain abnormal material has, however, been discovered during the present investigation which seems to throw some light on this subject. In the non-coiled anaphase chromosomes from slide 58-6d-3 it was noted that the threads are somewhat thicker in some places than the coiled threads at a similar stage in more normal

material. This suggests either that the threads are actually thicker in the former case or that the half-chromatids are more widely separated. Since the tertiary split is obvious only in a few places no very definite choice of the two alternatives can be made. Recent material of the same sort (slide 65-C7-c) is more useful in this regard. In this the tertiary split is clear in most regions of the anaphase chromosomes. Each half-chromatid is found to be quite widely separated from its neighbour and contorted by a more or less irregular waviness. It is this waviness which provides a very valuable clue to the problem of the minor spiral. There are several interpretations which might be placed on this condition. It is conceivable that these chromosomes illustrate a case of unravelling of both major and minor spirals, the former having been completely eliminated and the latter still represented by relic coils. Several factors contribute towards making this view rather untenable. In the first place the characteristic early stages of major spiralling are almost completely absent thus suggesting that it was not formed at all. Similarly, early stages which might be expected to show some signs of the minor spiral are, if anything, even less contorted than the anaphase chromosomes. Furthermore in certain regions of the anaphase chromosomes where the waviness is not too irregular some idea of the size of the supposed antecedent minor spiral can be obtained. This would certainly be large enough to distinguish, yet it was not observed. Finally, even a relic coil would not

be expected to be in one plane yet these loops are even where they are most regular. It, therefore, seems rather unlikely that this waviness is the result of the unravelling of a preceding minor coil.

It might also be urged that this waviness is indicative of a minor coil in the making. This cannot be definitely refuted except by pointing to failure to see any more evidence for a minor coil in the second division than in the first.

The third possibility is that this is an exaggerated case of what normally obtains in an anaphase chromosome. When the major coils are present the half-chromatids would be bound together to some extent and would thus achieve a certain amount of rigidity with which to resist bending. In the absence of the coils, however, there is nothing to hold the half-chromatids in such close association and their rigidity would be that much less and more bending would be found than is normally observed. A ready and apt explanation of why such bends should be produced lies in the observations of elongation. The medium within which the chromosome lies must offer some resistance to elongation which resistance might very well be expected to cause bending in the elongating chromonema.

This third hypothesis while fitting no better on its own merits than the second does fit better with what has already been learned about the behaviour of the chromosome

during this period. For this reason it seems advisable to accept it at least tentatively. If it is accepted another question must be raised. It has been seen that in most regions of the chromosome these minor bends are quite irregular but on the whole there seems to be some indication of a pattern. Bends tend to occur, if not at equal distances, at least within quite definite limits. There must be some reason for this. Few cytologists would care to say that the chromonema is homogeneous throughout its length. They would rather incline to the opinion that it is thick in some regions and thin in others. If, then, there is a resistance of the medium to the elongation of the chromonema the latter would bend more readily in the thin places than in the thick ones. From this it follows that the more regular the distribution of the thick and thin regions the more regular will be the bends. The concept that the chromomeres are thickened regions along the chromonema falls in with this idea extremely well, so that it may, with some justification, be supposed that, given enough resistance, bends will occur between all the chromomeres which while not spaced equally are nearly so.

If this is actually the case it follows that: <u>the minor spiral in Trillium is not a true spiral but a slight waviness due to the chromonema elongating through a resisting medium.</u> The mechanism is exactly that which causes the major coil but without the regulating presence of a pellicle.

Relational Coiling.

In the section on observations certain data on relational coiling (twisting) in first pollen-grain division were given. These data will now be analyzed in order to see whether they can offer any clue to the origin of relational coiling. Before this is done, however, it seems advisable to stress this point: any conclusions derived from this analysis must be considered as only tentative, particularly in view of the small number of data at present available.

Without recourse to involved analysis a brief examination of the data given in Tables XV-XIX elicits some useful information:

(1). The number of twists is less at metaphase than at prophase. This is particularly obvious in the data from material NW-5E-2 where observations were made at distinct stages. In the other two materials observations were made at all stages from mid-prophase to metaphase. Figures for these two stages, therefore, merge into one another and it is necessary to make a somewhat arbitrary distinction. This has been done in Table XXIII in which all nuclei with a total chromosome length of over 100μ have been called prophase and those with a length of less than 100μ , metaphase. When all three materials are taken together the mean number of twists per cell is 10.8 at prophase and 6.9 at metaphase. Each of the materials shows a similar decrease when considered individ-

ually. It is also obvious, from this table, that the decrease in the number of twists is directly proportional to the decrease in chromosome length, since the ratios of twists to lengths are practically the same at prophase and metaphase in each of the three materials.

TABLE XXIII.

The Ratio of the Number of Relational Twists per Cell to the Chromosome Length.

Material	Prophase (Above 100μ).			Metaphase (Below 100μ).		
	Mean length	Mean no. twists	Ratio	Mean length	Mean no. twists	Ratio
NW-5E-2	203.2	*7.6	0.037	81.5	4.2	0.052
65-M-65-P	129.5	14.7	0.114	76.3	9.2	0.120
63-RC	113.6	10.2	0.090	88.9	7.4	0.083
Total	446.3	32.5		246.7	20.8	
Mean	148.8	10.8	0.073	82.2	6.9	0.084

* Estimated from Table XV.

(2). From the numbers themselves, it would seem that there is some slight departure from randomness in the direction of twisting on either side of the attachment. Perfect randomness is shown, however, by material 65-RC but the numbers are so small in NW-5E-2 as to be meaningless in this regard. Materials 65-M and 65-P comprise the only group showing an apparent departure from randomness. Even in this case the deviation is not quite significant and the apparent discrepancy may reasonably be expected to be due to the small number of observations. The grouped data (Table XXIV) are well within the limits of probable deviation from randomness.

TABLE XXIV.

The Direction of Relational Twisting on Either Side of the Attachment.

Direction	R.R	R.L or L.R	L.L	R.U	L.U	U.U
NW-SE-2	2	1	1	5	5	10
65P 65M	7	3	5	14	5	3
63 - RC	3	11	2	14	9	5
Total	12	15	8	33	19	18

(3). It is also obvious that reversals in the direction of the relational twisting occur rather frequently within an arm. Some twenty-six cases of interstitial reversals have been observed in about 135 chromosomes. If only the arms which have two or more twists are considered there are 156 twists and 26 reversals. This is certainly a higher proportion than has previously been recorded.

Points (1) and (3) as outlined may be considered, in a general way at least, to be reliable and may be expected to hold regardless of the amount of data accumulated. It can scarcely be doubted that the number of twists decreases from prophase to metaphase or that reversals in the direction of twisting are frequent. More data may establish the frequency of reversals with more accuracy and the exact relation between decrease in relational coiling and chromosome contraction but it is unlikely to contradict the general conclusions.

On the other hand, the conclusions reached in point (2) are not so trustworthy. More data are necessary before the question of randomness across the attachment can be settled. If the totals for the three materials are considered they do fit a 1:2:1 ratio for R.R : R.L : L.L at the attachment but the fit is not extremely good. It may, therefore, be tentatively assumed that the twists are in random directions on either side of the attachment but the possibility that there is a slight tendency for them to be

the same cannot, at present, be eliminated.

Direct observation gives little clue to the time of development of the relational twist. Indications of twisting may be observed, however, at very early prophase of first pollen-grain division before uncoiling of the relic coil has occurred to any great extent. Since it can later be seen that twists are being eliminated with uncoiling and contraction, it seems unlikely that relational twisting is being developed during early prophase when the same processes that result in its elimination are occurring. It is also somewhat difficult to imagine how two threads coiled in parallel can become twisted about each other without first uncoiling. It, therefore, seems probable that relational twisting is developed sometime before or during the formation of the major coil and after or during production of the tertiary split. If it is developed during the time of splitting the simplest cause would be a rotation in the plane of cleavage; if after splitting, it may be a direct result of coiling a split thread.

With the present data it is impossible to do much more than speculate along the lines suggested by such information as is available. It may, however, be worth while examining the two possibilities mentioned above in the hope that some new angle of approach may be suggested thereby.

In Trillium the tertiary split becomes pronounced at first anaphase and presumably occurs either prior to or during coiling. If the plane of this split rotates, the half-chromatids will be relationally twisted and this twisting will appear at first pollen-grain division subject to such alterations as may be forced upon it by the major spiral. This may introduce many complexities and combinations of complexitites, depending on the pitch and direction of both coils and twists and the movements of the half-chromatids during coiling. Aside from the difficulties which must obviously arise from this angle there is also the difficulty of producing reversals. To produce them by means of a rotating cleavage plane demands that the split begin in several places at once, spiral in different directions and yet be so controlled that the cleavage would result in two absolutely equal threads. While such a mechanism may not be impossible, it is certainly improbable.

The second suggestion that spiralization is a more or less direct cause of relational twisting seems to have more in its favour. If two threads which are coiled together are to separate while coiled they must either coil independently or, as suggested by Kuwada (1927) they must twist about each other once in every gyre in the opposite direction to the coiling. As is particularly well illustrated by Dr. Hunter's asynaptic materials, the degree of randomness in the direction of coiling of the two threads is inversely proportional to the

degree of their association. The possibility that closely associated threads coil independently can, therefore, scarcely be considered. It must, then, be assumed that the sister chromatids of type (1) asynaptic material coiled in the way suggested by Kuwada. Such threads could then separate while coiled but if uncoiling occurred before separation, they would, unless they had separated far enough while coiled to avoid entanglement, become relationally twisted about each other. The half-chromatids formed by the tertiary split do not separate until they have become uncoiled. Furthermore, if by the time spiral coiling occurs the tertiary split were well enough developed to allow the half-chromatids to twist about each other, spiralling should produce a relational coil visible as such during prophase of the first pollen-grain division. There is no doubt that such a mechanism could produce a relational twist but whether or not its properties would be the same as that of the observed must yet be considered.

In the first place such a coil must always be in the opposite direction to the spiral that produced it. Whether or not this is so in the case being considered cannot be determined from the present information. This would necessitate a comparative analysis of the direction of relic and relational coiling at a stage when both could be done accurately. So far this has been found to be impossible.

In the second place, since the direction of spiral coiling is approximately random on either side of the attachment

it follows that the direction of relational twisting must also be random on either side. From the data it can only be said that this is probably true of the observed relational twist but definite conclusions in this regard can not be drawn.

In the third place, since interstitial changes are frequent in the major spiral, they must be similarly frequent in the relational coil. This seems to hold. Table XXV gives the frequency of changes in direction in the major coil expressed as the ratio of the number of changes per cell (minus those assumed to be associated with the attachments) to the number of gyres per cell. These figures are the means of the means from Tables VII, X, XI, XII and XIII. The proportion of reversals in the relational twist is expressed as the ratio of the number of reversals to the number of twists observed, eliminating, of course, all chromosome arms which had less than two twists. These two ratios are almost exactly the same.

It may be unwise to attempt to read too much into these figures but it does seem rather probable that they indicate a general connection between spiral coiling and relational twisting and more specifically, a direct relation between changes in direction in the two.

If for every gyre of the major spiral there is also one twist in the relational coil, it might be considered that the number of twists would be the same as the number of gyres, at

TABLE XXV.

A Comparison Between the Frequencies of Changes in Direction in the Major Spiral and the Proportion of Reversals per Twist in Relational Coiling.

The Major Coil of Meiosis		
Mean no. gyres per cell	Mean no. interstitial changes per cell	No. changes / No. gyres
181.1	30.8	0.17
Relational Twists of First Pollen-Grain Division		
Total no. twists	Total no. interstitial reversals	No. reversals / No. twists
156	26	0.16

least before contraction had eliminated some of the twists. This would undoubedly be so were it not for changes in direction which, in turn, cause reversals in the relational coil. Opposing twists in relationally coiled threads unlike changes of direction in the gyres of a spiral would be expected to cancel each other as soon as the two threads became independent enough for such cancellation to occur. Furthermore,

twists of opposite directions which were close together would do so sooner than those which were farther apart since the loops between the former would fall apart easier than between the latter. It is an observational fact that twists in opposite direction are almost invariably separated by a relatively long untwisted region. It is, however, possible to estimate the number of twists per cell which any anaphase set of chromosomes may be expected to yield. The probable number of cancellations can be obtained by considering the number of gyres on either side of a change in direction; e.g., if there are 4 gyres on one side and 5 on the other a single relational twist would be expected. This calculation has been made for several anaphase cells from various materials which have been analyzed for number of gyres and changes in direction, with the result that a derived relational coil is expected to have 25-30 twists per cell. In the present pollen-grain material the total length of the five chromonemata has been reduced to about one-half that at anaphase. There is evidence that the number of twists decreases directly with a decrease in length. Therefore the number of twists should be 12-15 which is within the range of the observed numbers. On the whole, however, these numbers are slightly higher than the observed particularly when it is remembered that the calculation by which they were obtained does not allow for any reversals still present. There are two possible explanations for this: (1) while a tendency for half-chromatids to twist about each other during

coiling has been assumed it need not follow that this twisting always occurs. (2) If the tertiary split was not complete prior to coiling the number of relational twists would be reduced. Both of these conditions might be expected to occur and, if so, they would provide an adequate explanation for occasional mid-prophase chromosomes which show no relational twisting at all.

The hypothesis is, therefore, tentatively proposed that the relational coil observed in first pollen-grain prophase is derived from the preceding major spiral through a twisting developed during coiling. That twisting has a pitch and direction which compensate for the major spiral. In the specific case under consideration, this hypothesis is adequate in that it gives a satisfactory explanation for the observations. It must be remembered, however, that the volume of data is too small to allow for unqualified interpretation and some information which is necessary in ascertaining the validity of this hypothesis is not at present available.

A Unified Hypothesis of Spiral and Relational Coiling.

As is suggested by this sub-heading, the following discussion is frankly concerned with hypothesis rather than with fact. Before considering the hypothesis, however, it may be well to review briefly the more outstanding points of the previous discussion of coiling. From a consideration of the observations themselves as well as of their analyses, the

following conclusions have emerged:

(1). The development of the major spiral is accompanied by an elongation of the chromonema and a slight contraction of the chromosome.

(2). No true minor spiral has been observed but the anaphase chromonema does exhibit a slight waviness the loops of which are irregular within limits.

(3). The direction of the major coil may change both at the attachment and within the chromosome arms.

(4). The major coil is retained through second division and does not uncoil until first pollen-grain division prophase.

(5). The chromatids of the unravelling chromosomes are relationally twisted.

(6). The number of twists is being diminished during contraction and uncoiling.

(7). The relational coil reverses its direction with the same frequency as the major spiral.

There is little reason to doubt the factual nature of any of these statements. They may, therefore, be considered to form a relatively sound foundation for hypothesis.

Several hypotheses have been proposed in the earlier portions of this discussion to correlate the observations on various phases of the coiling problem. These hypotheses may be summarized as follows:

(1). The major coil is directly due to the elongation of the chromonema within a limiting pellicle.

(2). An analysis of the changes in direction of this coil indicate that they are due to points of interruption which are of three kinds: (a) the attachment, (b) chiasmata and (c) some factor the effectiveness of which is directly proportional to the number of gyres.

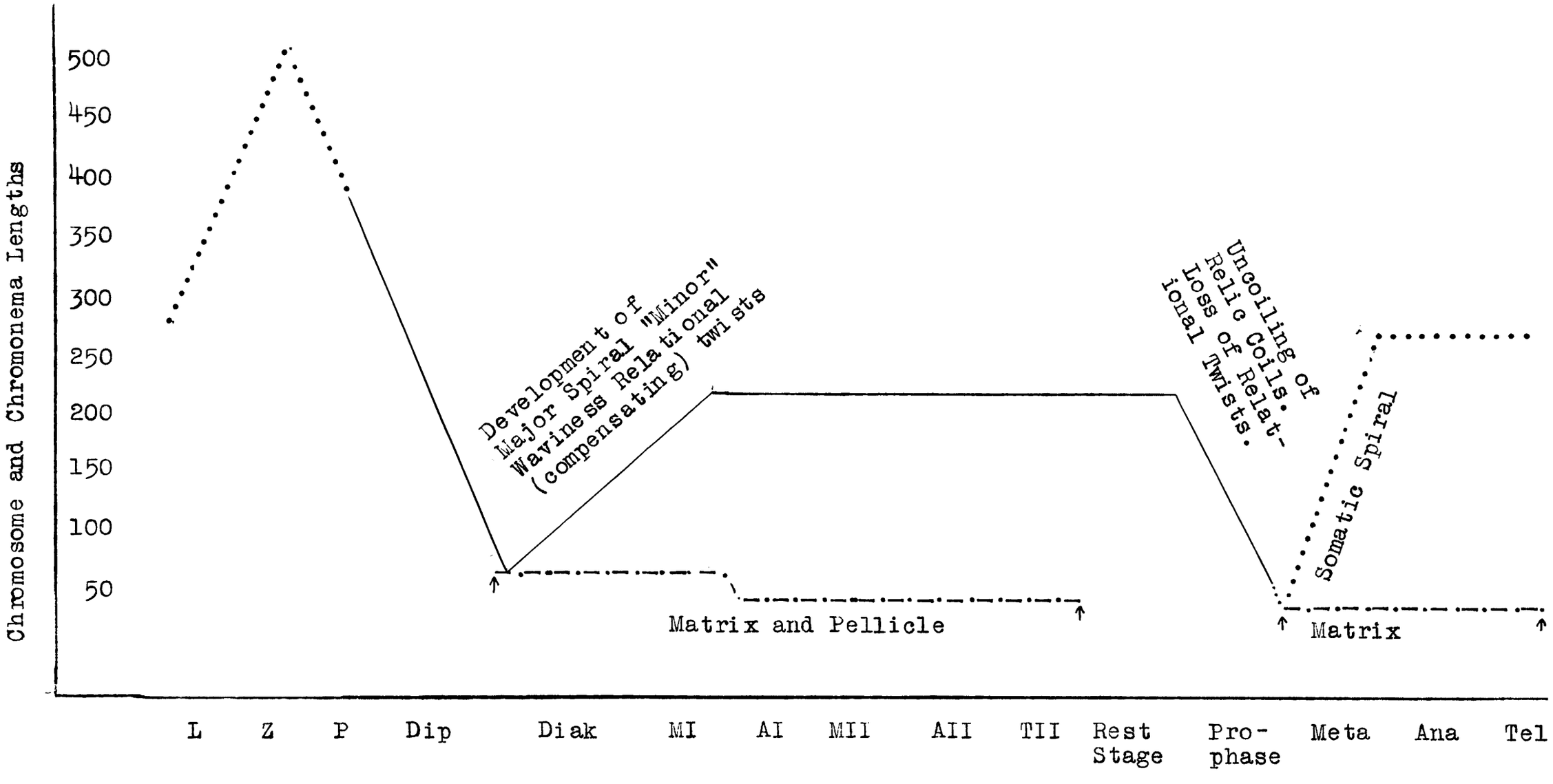
(3). The "minor spiral" is not a true spiral but a waviness caused by the elongation of a longitudinally heterogeneous chromonema through a resisting medium.

(4). The relational coil of first pollen-grain division is directly due to the tendency of two halves of a split thread, coiling in the way suggested for formation of the major spiral, to twist about each other in a manner which compensates for the major coil.

The summation of these hypotheses equals a single unified hypothesis of coiling which may be set forth thus: <u>The fundamental factor in coiling is the elongation of the chromonema. The types and properties of the coils thus formed are determined by the structure of the chromonema and the</u>

conditions under which elongation occurs. Obviously this hypothesis may be invalid. It has been derived from the data by the following process: Certain conclusions have been drawn from analyses of the data; these conclusions were then correlated by means of several hypotheses concerned with separate phases of the problem; and finally, these separate hypotheses were in turn correlated into one unified hypothesis concerning the whole. Errors may have been made in any of these steps and the chances of error increase greatly with each step taken. It is, nevertheless, a fact that this hypothesis can adequately explain such observations as have been made and it is, therefore, regardless of its probable validity, of considerable use since it serves to correlate and emphasize the observations which would otherwise be a meaningless accumulation of data, the possible importance of which might very well be entirely overlooked. It is furthermore true that this hypothesis, in common with all such theoretical structures, points clearly to certain gaps in our knowledge which might otherwise be unsuspected.

In regard to the validity of the hypothesis it must be pointed out that no adequate assessment of its truth can be made until all gaps are filled nor can its general application be evaluated until similar studies are carried out on other organisms particularly those which differ from Trillium with respect to coiling.



Text fig. 19. A graphical representation of the relation between coiling and chromosome and chromonema length changes as suggested by the proposed hypothesis.

CONCLUSIONS AND SUMMARY.

(1). In general the structure of the chromosomes of Trillium erectum L. during meiosis and the first pollen-grain division has been found to be as described by Huskins and Smith (1935).

(2). The attachement at first metaphase and anaphase and at metaphase of pollen-grain division is a region of 2-3μ in length, differentiated into central and terminal regions which show different degrees of attraction for associated strands.

(3). The major spiral begins development as a more or less regular waviness apparent at early diakinesis and gradually assumes the form of a spiral which reaches its maximum compactness at first anaphase.

(4). No true minor spiral has been observed but the coiled anaphase chromonema exhibits a slight waviness along its gyres.

(5). The major spiral remains unchanged throughout second division and becomes unravelled in the prophase of the first pollen-grain division.

(6). The chromatids of the first pollen-grain division are wound about each other in a relational twist or coil

the number of twists of which decreases with prophase contraction.

(7). An analysis of the length changes in the chromosome and chromonema during formation of the major spiral shows that the chromonema about doubles its length while the chromosome probably contracts slightly. It is suggested that the outer surface of the chromosome is a sheath or pellicle and that elongation of the chromonema within this causes spiralization.

(8). Analysis of the changes in direction of the major spiral indicate that they are probably random at the attachment, at chiasmata and at undefined points, the number of which is proportional to the number of gyres. Such a condition would be expected on the suggested mechanism of coiling.

(9). The relational coil can reverse its direction both at the attachment and within the chromosome arms. Since the proportion of these reversals to the number of twists is the same as the frequency of the changes in direction of the major spiral, it is suggested that the two types of coiling are related.

(10). It is suggested that the relational coil is formed during development of the major coil due to the tendency of the half-chromatids to twist about each other during the process in such a way as to compensate for the spiral.

(11). It is further suggested that both types of coiling are directly due to the elongation of the chromonema between diakinesis and first anaphase in that elongation of a split thread within a limited space would result in the simultaneous production of a spiral and relational coil.

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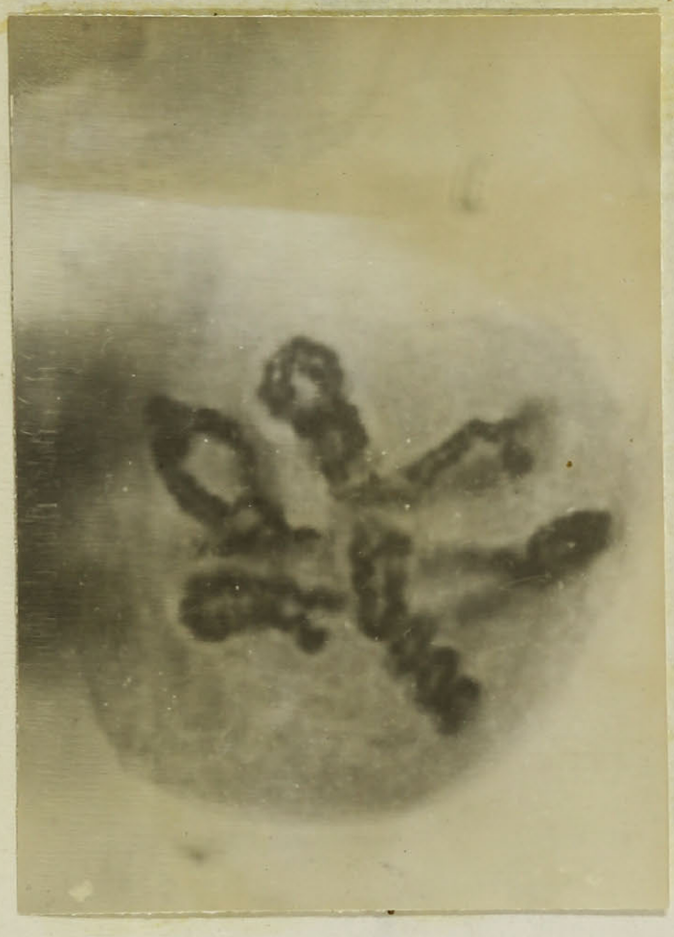
PLATE I.



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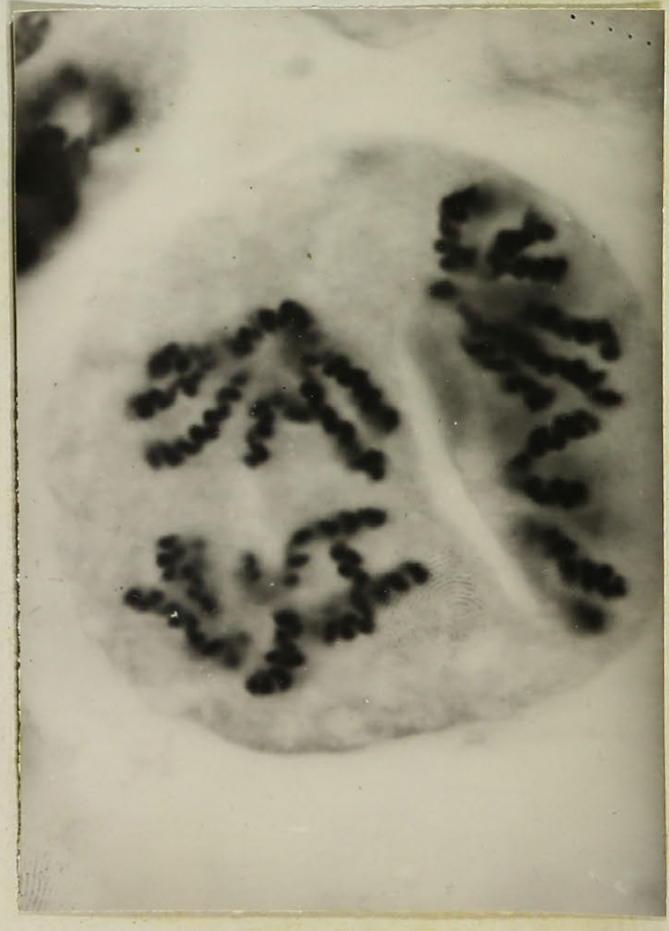

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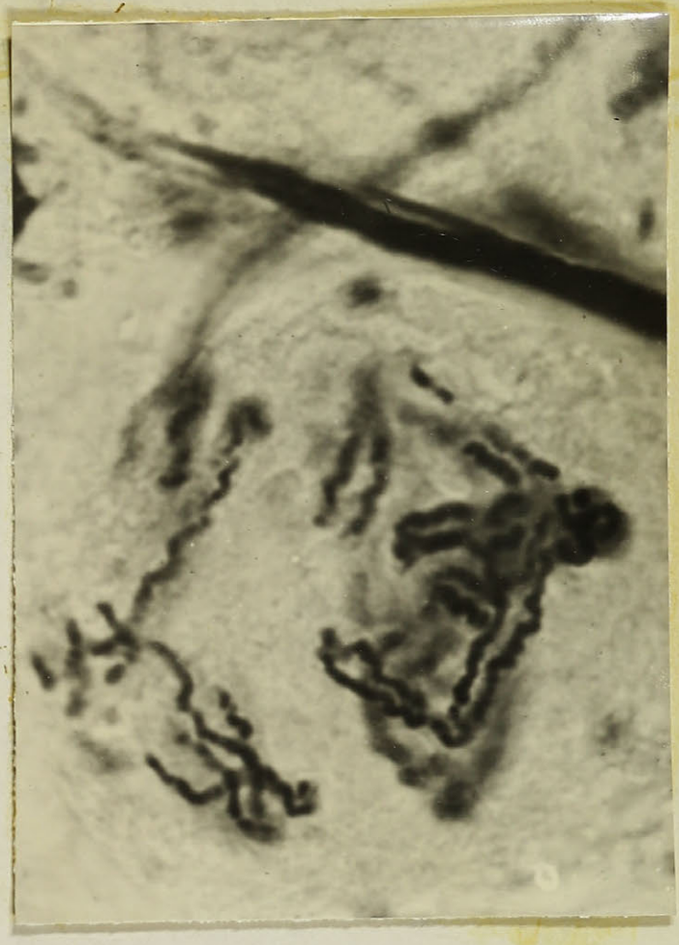

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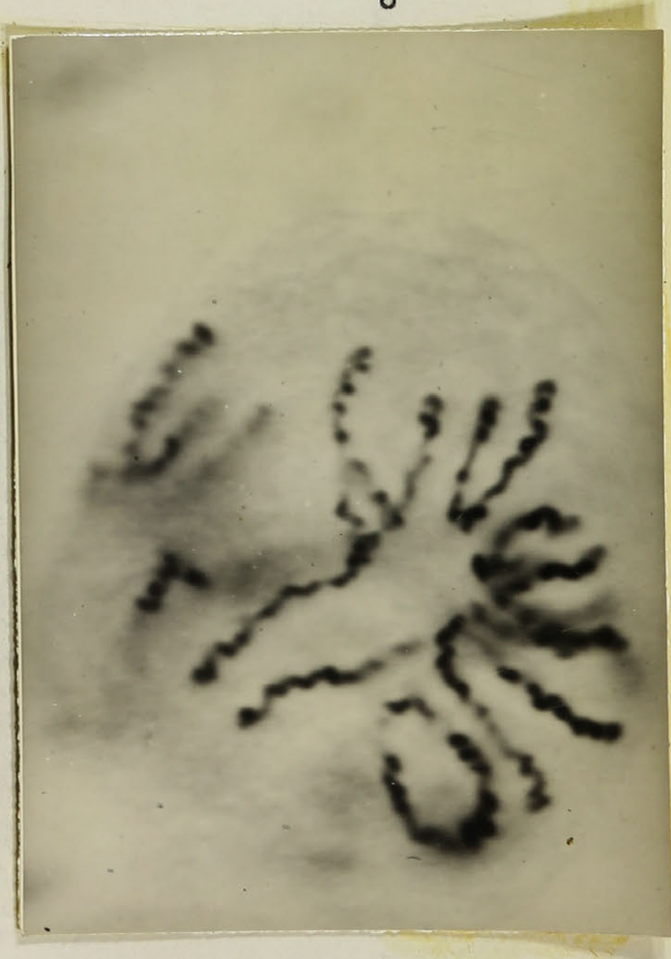

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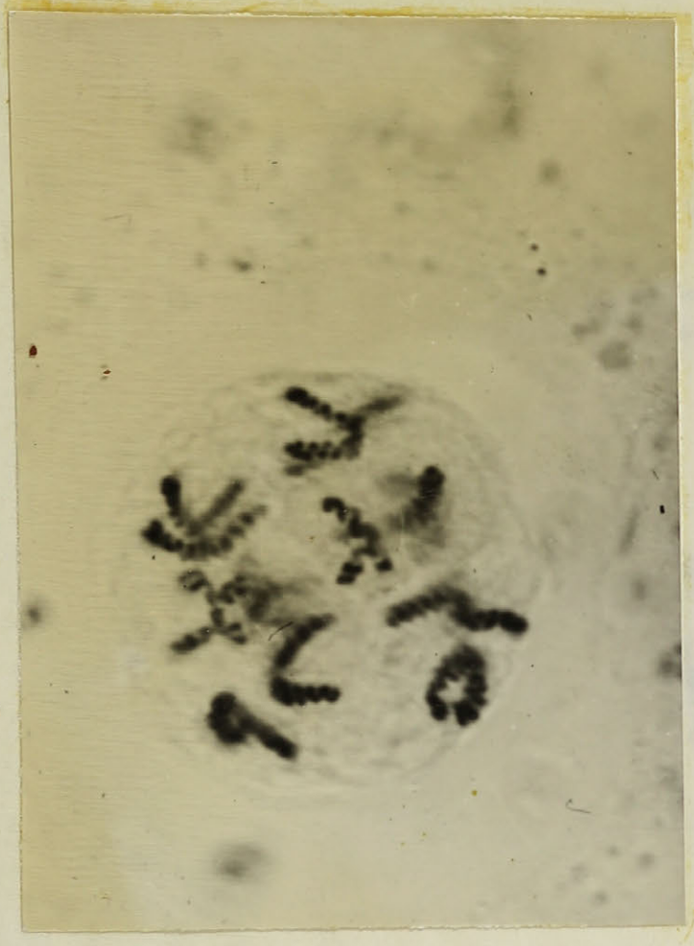

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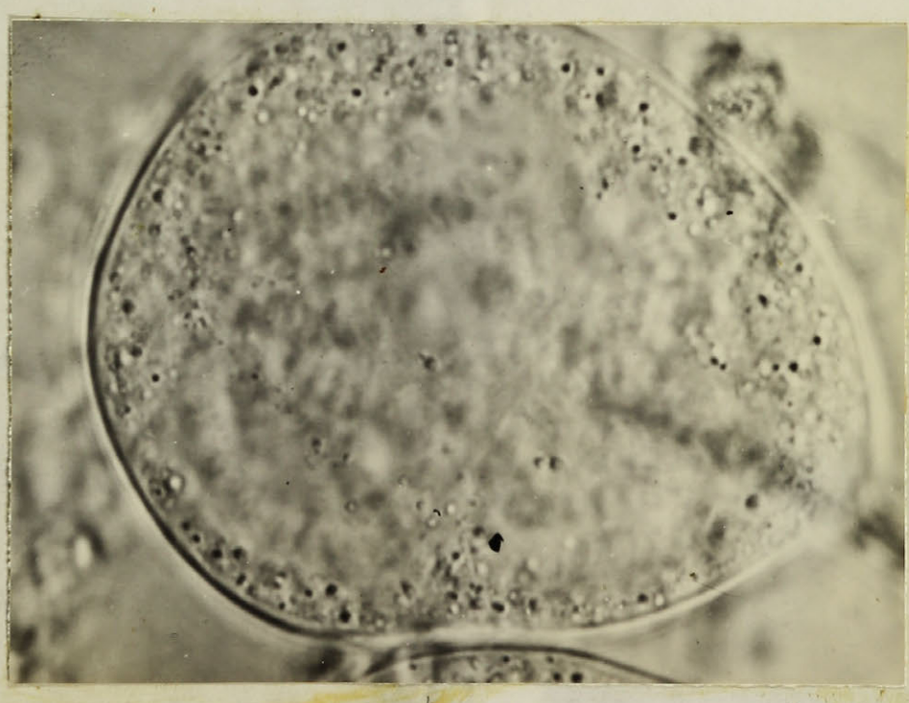

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PLATE IV.

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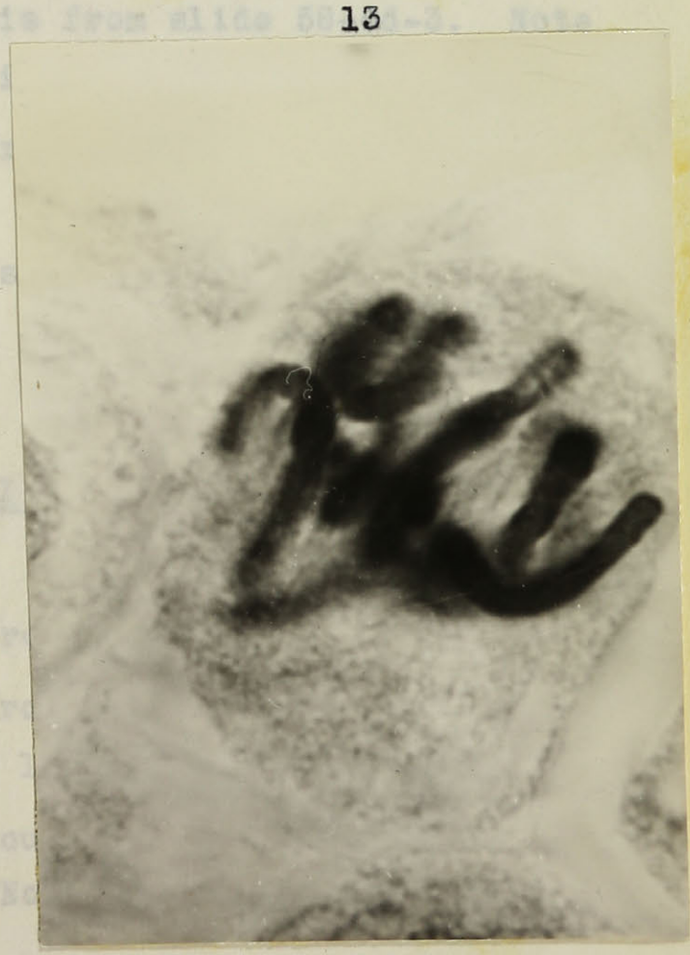

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Description of Plates.

The magnification of all figures is approximately 2000x.

Plate I.

Fig. 1. A cell at early diakinesis from slide 58-5d-3. Note that coiling has not begun in most chromosomes.

Fig. 2. A cell at late diakinesis from slide 58-5d-3. The major coils are partially developed.

Fig. 3. A cell at early first anaphase from slide 58-5d-3.

Fig. 4. A second anaphase cell from slide 58-5d-3.

Plate II.

Fig. 5. A cell at late diakinesis from slide 58-6d-3. Note the absence of major coiling.

Fig. 6. A first anaphase cell from slide 58-6d-3. No major coils have developed.

Fig. 7. A cell at mid-diakinesis from slide 65-L2-a.

Fig. 8. A second metaphase cell from slide 65-L2-a. cf. Text-fig. 9.

Plate III.

Fig. 9. A first anaphase cell from slide 58-2E-1.

Fig. 10. A first anaphase cell from slide 65-C7-c showing a wide tertiary split and lack of major coiling.

Fig. 11. A first anaphase cell mounted in 4% can sugar without fixation or staining. Note the chromonema spirals.

Plate IV.

Fig. 12. A prophase cell from first pollen-grain division in material 63-RC.

Fig. 13. A metaphase cell from first pollen-grain division in material 63-RC. Note the attachment region.

Fig. 14. A cell at early metaphase of first pollen-grain division. Note the four-partite condition of the chromosomes.

Fig. 15. Late metaphase of first pollen-grain division showing a few gyres of a somatic spiral.