STUDIES ON THE CHROMOSOME SPIRALIZATION CYCLE IN *TRILLIUM*

- by -

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INTRODUCTION

"Broadly speaking, the sum-total of the concepts involved in any material phenomenon is a constant (P); and if we simplify or diminish the complexity of the substance or matter units (M) reckoned with, we must correspondingly complicate or increase the behaviour factor (B). That is, since the phenomenon is a function of both M and B, we may write

$$P = f(M,B)$$

and any increase in the complexity of M involves a corresponding simplification in B, and vice versa."
{Bridges (Alexander, 1928)}

This formula no doubt applies to the concepts concerning any field of investigation at a given time. But, since M and B are both variables, it would seem from a survey of the literature over an extended period that P must necessarily be increasing. So it appears to most biologists interested in relating the problems of genetics with chromosome structure and behaviour. Not only to these people, at close range, does P appear to be increasing, but the rapid advances in the field of investigation are even attracting the attention of those not fundamentally interested in this branch of biology. It is becoming

increasingly apparent that the not so long ago "upstart science" of genetics has begun to have important implications in other fields of endeavour, however secure the specialists in those fields may have considered themselves in their self-complacent isolationism. Thus, modern genetics and chromosome cytology are beginning to have an integrating value in modern biology, and may even come to be instrumental in closing the ever-narrowing gap between living and non-living, between biological and physical sciences.

Few biologists today would venture to deny that the study of genetics and the ultimate acceptance of the chromosome theory of heredity has materially changed and advanced our concepts of the evolutionary processes. The structure and behaviour of chromosomes thus comes to have a general significance for all branches of biology, and it is, therefore, not too presumptuous to hope that the present investigation of chromosome mechanics may add something, be it ever so little, to the ultimate goal of rendering biology as a whole more intelligible.

REVIEW OF LITERATURE

Introduction

In spite of the fact that the spiral structure of chromosomes was observed by Baranetsky in 1880, with a few notable exceptions this important phase of cytology was largely neglected for over forty years (Janssens, 1901: Bonnevie, 1908, 1911; Schneider, 1910; Vejdovsky, 1912). Chromonemata and spirals were frequently described or figured (Janssens, 1901; Pinney, 1908; Digby, 1910; van Herwerden, 1910; Dehorne, 1911; Wilson, 1912; Mohr, 1914; Wenrich, 1916; Lee, 1920, 1924; Kuwada, 1921; Litardiere, 1921; Newton, 1924, and others) but in most cases only at prophase or telophase. Schneider (1910) apparently recognized the chromonema as a persistent structure, but most of the workers who studied the chromosome cycle extensively enough to recognize both chromonema and spirals considered them to be replaced at later stages by other structures. Many authors (Balbiani, 1881; Strasburger, 1882; Carnoy, 1884; Bolsius, 1911; Alverdes, 1912) described a disc structure, which considerably confused the issue, while others, who actually observed spiral structure, tried to explain it away as an artifact (Sharp, 1913), or considered what we now recognize as fixation artifacts. e.g., alveolation (see Sharp, 1934, p. 140; Darlington and LaCour, 1938), to be typical of chromosome structure (Chamberlain, 1925).

That the chromonema is a permanent structure and maintains its integrity even during resting stage and throughout spiralization was first demonstrated by Kaufmann (1925, 1926 a and b) in Tradescantia and Podophyllum, and by Kuwada (1926, 1927) in Vicia. These papers seemed to stimulate new interest in the subject, and literature in the field of chromosome structure and spiralization has accumulated during the last fifteen years to such an extent that a complete review of all papers on the subject is out of the question here. Most phases of microscopic and submicroscopic chromosome structure have been dealt with in recent reviews by Darlington (1937), Kaufmann (1936), Huskins (1937), Bauer (1938), Frey--Wyssling (1938), Geitler (1938a), Straub (1938), Nebel (1939 a and b), Kuwada (1939, 1940), and Kuwada and Nakamura (1940). The present review will confine itself largely to papers rather intimately related to the problem under investigation, namely, the relationship between chromosome, chromonema and chromatid length changes and the spiralization cycle, with special reference to the development of the major and relational coils and the origin and frequency of changes of direction in both.

Theories of Spiralization.

As a natural concomitant of the purely morphological study of chromosome structure cytologists began to speculate and inquire into the mechanics of spiralization. Proponents

of the various theories of coiling can be divided into two categories; those who regard the matrix as the dynamic force, with the chromonema playing a minor role, (Bridges, (Alexander, 1928); Sax, 1930; Sax and Humphrey, 1934); and those who regard changes within the chromonema as of paramount importance (Kuwada, 1927, 1935; Belar, 1928, Darlington, 1935; Huskins and Smith, 1935; Wilson and Huskins, 1939; Nebel, 1939a).

Bridges considers that "the gene string and its immediate envelope of gel . . . becomes suspended like an elastic rod in a fluid colloid within a pellicle". Surface tension would tend to reduce the narrow, cylinder shaped chromosome to a more spherical one. The thread would first be forced into a zig-zag and gradually into a regular spiral enclosed within a pellicle. Bridges also mentions mutual repulsion, syneretic contraction of the pellicle and forces within the chromonema comparable to those involved in tendril coiling as possible additional mechanisms.

Sax (1930), who studied meiotic coiling in Secale, and Sax and Humphrey (1934), in Tradescantia, also conclude that coiling is due to a differential rate of contraction between the chromosome, which shortens, and the chromonema, which retains its original length by coiling (the latter applies to Secale). According to Kuwada (1926), Vejdovsky (1912) considered that chromosome contraction could give

rise to a spiral structure, presumably the chromonema maintaining its original length.

Kuwada (1927) suggests that chromonema contraction forces the embedded chromomeres into a zig-zag and then into a spiral, but later (1935 and 1937) combines the postulated anisotropic swelling and internal twisting of the chromonema with a matrical contraction to explain coiling.

Darlington (1935) introduced an assumed "molecular spiral" into a scheme of coiling in which a torsion is set up by a systematic molecular asymmetry within the gene string. This primary torsion, which is subject to unitary control within each arm, results in a visible coiling in the opposite direction. The torsion hypothesis, which the Darlington school (Darlington, 1936b, 1937, 1939, 1940a,b; Mather, 1938; Frankel, 1940) still upholds, fails to explain randomness of coiling and interstitial changes of direction.

Nebel (1939a) has modified Darlington's molecular coil to allow intrabrachial changes of direction. He postulates that the "genonema" is composed of flat submicroscopic "nemameres" (intermediate in size between molecules and chromomeres). Various attractions and repulsions and an "external polarizing force" act on the nemameres to produce contraction, elongation and helication.

Huskins and Smith (1935) presented a working hypothesis of spiralization based on the concept of heterogonic growth. If growth in thickness occurs in the half chromatids which are twisted about each other, it will necessarily be in the form of a spiral. Syneresis in the "newly laid down extra-chromatin" will give rise to an unequal tension on alternate sides, resulting in a zig-zag or spiral. The more recent work of Wilson and Huskins (1939) demonstrating an elongation of the chromonema during the formation of the major coil seems to render this hypothesis, as originally presented, no longer tenable.

Length changes during the Spiralization Cycle.

Meiotic prophase contractions prior to formation of the major coil have been measured by several authors (Gelei, 1922; Belling, 1928, 1931; Dark, 1934; Sax and Sax, 1935; Koller, 1936), who have calculated a contraction ratio on the basis of mass-stained metaphase chromosomes. Matsuura (1934) and Naithani (1937) have compared meiotic chromosome length with that of its spiralled chromonema. Both meiotic prophase stages and later coiled chromonema have been measured by Wilson and Huskins (1939) and Manton (1939). Somatic chromosome length changes have received considerably less attention (Belar, 1926; Sax and Sax, 1935; Upcott, 1936; Patau, 1937), although somatic metaphase chromosome sizes are known for many species and available for many more from published illustrations.

Belling (1928, 1931), who seems to have been the first to make careful measurements during meiotic prophase in plants, found, contrary to general opinion, that expansion occurred between leptotene and pachytene. In both Lilium and Aloë the end-to-end length of metaphase chromosomes was only one tenth that calculated for pachytene chromonemata. He considered that the chromonema could contract without zig-zagging to one third of its original length, and that the rest of the shortening was due to spiralization. Dark's (1934) results in Bellevalia and Gelei's (1922) on Dendrocoelum agree rather closely with Belling's. The former found a contraction by metaphase to one eleventh of the pachytene length and Gelei to about one ninth between leptotene and oogonial metaphase. Measurements from Smith's (1941) drawings of Diprion oogonia indicate a contraction ratio of approximately 15:1 between pachytene and first metaphase. A much lower ratio (approximately 4:1) was reported by Koller (1936) in two genera of Marsupials. Meiotic prophase contractions reported by Sax and Sax (1935) for Vicia, Tradescantia, Lilium, Secale and Allium all fall within the limits already given. The average reduction between pachytene and meiotic metaphase is about 9:1. However, they report that, according to a personal communication from McClintock, the contraction ratio in Zea Mays may be as great as 15:1, although her published figures (1933) show only about 11:1. A still greater ratio (18:1) was recorded between leptotene and

second anaphase in Osmunda (Manton, 1939, aceto-carmine series). The above contraction ratios are all calculated on the basis of end-to-end chromosome length and do not take into account the spiral structure characteristic of meiotic chromosomes after diakinesis.

Contraction ratios during mitosis in plants seem to be generally less than at meiosis, according to the work of Sax and Sax, who report an average reduction of about 3:1 in root tip cells and possibly a somewhat greater reduction in microspores (5:1 in Tradescantia). Patau (1937) found a ratio of 5.5:1 between prophase and anaphase in Collozoum, but Belar (1926) reports contraction ratios varying from 2.5:1 to 33:1 in Aggregata Eberthi. Evidently the contraction mechanism is highly variable in the Protozoa, for Cleveland (1938) found a constant degree of spiralization throughout the mitotic cycle in two species of flagellates. Berger (1938) estimates that mid-prophase compound chromosomes in the ileum of Culex are between ten and fifteen times their length at metaphase. This is considerably less than the difference between salivary chromosomes of Drosophila and the normal somatic metaphase (about 70 to 100 times, Cooper, 1938). However, it seems that such extreme elongation is an irreversible process in contrast to the usual

elongation at prophase stages. Elongation during the transition from mitotic to salivary chromosomes apparently is not uniform throughout, for Hinton (1940) has found that an inert region in the left arm of the second chromosome is represented in the salivary chromosome by a relatively much shorter region. Berger has made the interesting speculation that if all the achromatic regions were removed from a salivary chromosome, its length would approach that expected on the basis of the normal length ratio of prophase and metaphase stages.

Matsuura (1934) and Naithani (1937) have measured the lengths of spiralled chromonemata at meiosis and compared them with chromosome lengths at the same stage. The ratio of chromonema to chromosome length was 9.8:1 at first metaphase in Trillium kamtschaticum and 6.5-7.3:1 at second metaphase of Hyacinthus orientalis. Unfortunately prophase stages were not measured by these authors and contraction ratios are therefore not available.

The important relationship between chromonema elongation and major coiling was first shown by Wilson and Huskins (1939), who measured chromosome and chromonema lengths from early diakinesis to first anaphase of Trillium erectum. During this period the chromonema about doubled in length, while the chromosome changed relatively little. The ratio of chromonema to chromosome length at first anaphase in their standard material (1) was about 3.2:1 (using lengths calculated from the formula for a spiral). Several previous

workers have considered that the gene thread or chromonema maintained a constant length (Bridges (Alexander, 1938); Sax, 1930; Naithani, 1937; and Manton, 1939, the latter during mitosis only).

Similar measurements of chromonemata before and after spiralization and in mitotic metaphase and anaphase chromosomes have been made by Manton (1939) on Osmunda. Since leptotene and pachytene were measured, but no stage corresponding to diakinesis in Trillium, it seems quite probable that the shortest stage was missed. We can not, therefore, safely conclude that elongation does not accompany spiralization in Osmunda. The chromonema to chromosome ratio at somatic metaphase was 5.6:1 and at meiotic stages varied between 4:1 and 6.2:1 (aceto-carmine series).

In view of the range in contraction ratios in the various organisms mentioned above, Darlington's (1937) contention that the degree of spiralization must be influenced by genotype seems not unjustified, but it is abundantly evident that environmental factors also are concerned (Matsuura, 1937c; Sax, 1937; Huskins and Wilson, 1938; Giles, 1939; Barber, 1940; Matsuura and Haga, 1940; Huskins and Newcombe, 1941).

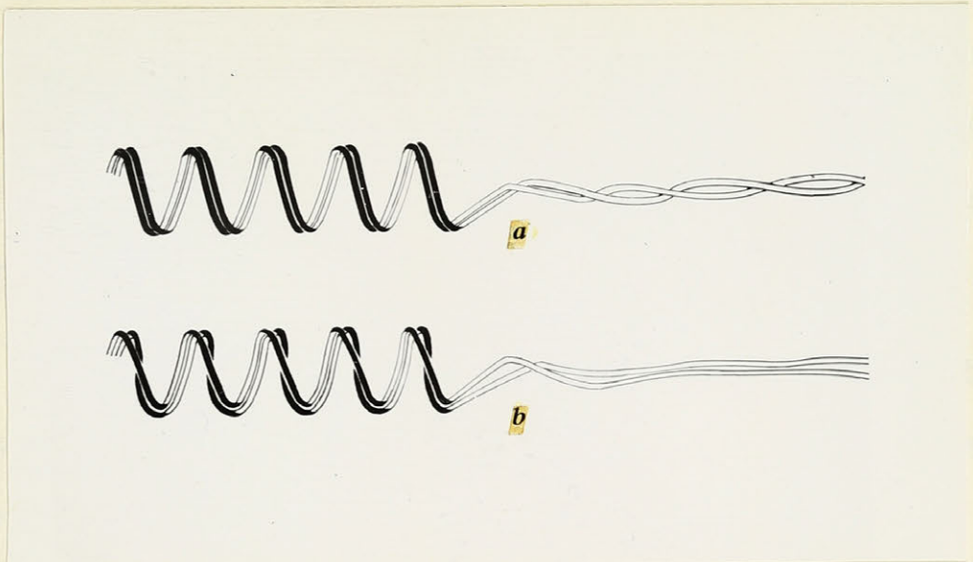
It seems rather surprising that so many cytologists should have realized the importance of accurate analyses of chromosome and chromonema length changes without having

made complete analyses throughout the whole spiralization cycle of both meiosis and mitosis in a variety of organisms. However, careful measurements of all stages, even of meiosis, have not yet been made in any organism, although Manton has come nearer this goal than any previous investigator. In our opinion, therefore, the data to be presented should be of particular value in that they are the most extensive study yet made, in any organism, of the cyclic chromosome changes occurring between early meiotic stages and the succeeding somatic anaphase.

Structure of Meiotic Chromosomes.

The spiral structure of meiotic chromosomes is now generally accepted, and has been demonstrated for a large number of organisms by many investigators. (For lists of literature on spiral structure see Lorbeer, 1934, Table 4; Geitler, 1938a, p.61; and the recent reviews referred to on page 4).

Kuwada (1939) describes two types of spirals: (1) orthospirals, which are formed when two separate strands with a free end are coiled together; (2) anorthospirals, which result when two strands with both ends fixed are coiled together and in consequence have a twist compensating for each gyre of the spiral (see Text, fig.1). Orthospirals are interlocked and cannot be separated without untwisting; anorthospirals are independent and can readily be pulled apart or fitted into each other. When a pair of orthospirals is straightened out, it will give a pair of relational twisted strands, while anorthospirals will give two independent strands. The term "paranemic" (para = beside) will be used instead of anorthospiral, since it is simpler and its implications are clear. Instead of orthospiral, "plectonemic" (plektos = twisted) will be used, as it has the advantage of indicating the relationship of the strands, both when in the major coil and when straightened out into the relational coil twisted in the same direction to which, as will be shown, it gives rise.



Text-Figure 1.

(a) A plectonemic coil ("double-stranded" ortho-spiral).

(b) A paranemic coil ("double-stranded" anortho-spiral). In the former the component halves are interlocked and form a relational coil when the gyres are partially straightened out. The two spirals of a paranemic pair are free to separate and therefore give independent strands when straightened out.

Although cytologists are now agreed as to the reality of the major spiral at metaphase and anaphase of both meiotic divisions, there is still considerable controversy as to its mode of origin, and its finer structural details (i.e., the minor spiral and tertiary split).

It is fairly well established that the chromatids separated by the secondary split form twin coils (Kuwada's anorthospiral, our paranemic spiral) whose gyres are free to fall apart laterally, forming a V or X-shaped dyad at first anaphase, (Kuwada, 1927; Maeda, 1928; Sax, 1930; Shinke, 1930, 1934; Kaufmann, 1931, 1936; Taylor, 1931; Taun, 1931; Nebel, 1932b; Kuwada and Nakamura, 1933; Koshy, 1934, 1937; Matsuura, 1934; Sax and Humphrey, 1934; Darlington, 1935; Huskins and Smith, 1935; Sax, 1935; Chandler et al, 1937; Haga, 1937; Naithani, 1937; Warmke, 1937; Huskins and Wilson, 1938; Manton, 1939; Hillary, 1940; Iwata, 1940). A few authors have reported that in Tradescantia, the sister chromatid spirals at first division are more or less interlocked (partially or wholly plectonemic) (Darlington, 1937, fig. 137d; Kuwada and Shinke (Kuwada, 1938); Toyohuku, 1938; Matsuura, 1938b), while Matsuura (1940) is of the opinion that in Trillium kamtschaticum the sister chromatids are plectonemic at early stages of first division and paranemic at late anaphase. He postulates a mechanism of crossing over, dependent upon the conversion of the plectonemic spiral to the paranemic by a process of breakage and reunion in

every interlocked gyre. In all the above cases there need be no serious objections on the grounds that separation will be hampered by the interlocked spirals, for in Tradescantia the major coil is unravelled at interkinesis, and in Trillium the paranemic condition is attained by the end of first anaphase.

A small gyred-coil within the chromatids of the major spiral, and variously known as "primary coil", "secondary coil", "double-coiled spiral" or "spiral within a spiral", was first described by Fujii (1927). The term "minor spiral", first introduced by Huskins and Smith (1935), seems to lead to less confusion than some of the other terms and is self-explanatory.

Following Fujii's discovery of the minor spiral, a number of papers confirming the "double-coiled spiral" appeared (Shinke, 1930, 1934; Ishii, 1931; Kuwada, 1932; Nebel, 1932a; Kuwada and Nakamura, 1933, 1934a and b; Kato 1935; Darlington, 1935; Kato and Iwata, 1935; Matsuura, 1935a; Sax, 1935; Sax and Sax, 1935; Oura, 1936; Makino, 1936; Takamine, 1937; Hillary, 1940). Huskins and Smith (1935) found no evidence for a regular minor spiral in Trillium, but stated that "each half-chromatid is, however, irregularly waved or twisted, and may possibly with some treatments be found to constitute a minor spiral". When Huskins (1937) again questioned the reality

of a regular minor spiral, Kuwada (1938) replied that "the question of the spiral form, whether a regular spiral or a mere corrugation, is not a fundamental problem, but only a consequence of secondary importance, resulting from the difference in the condition in which spiralization takes place".

The question of the time of occurrence of the longitudinal split in meiotic chromosomes has long attracted the attention of cytologists, but early observations must be largely discounted now, since they did not take into account the spiral nature of the component chromonemata. Observations of the tertiary split in spiralled meiotic chromatids have been reported or figured by many investigators during the last ten years (Table I). The majority of these have merely recorded the existence of the split without reference to the coiled relationship of the resultant half-chromatids. However, it is probably significant that all those who do make the relationship clear indicate that the half-chromatids form a plectonemic spiral (see also Kuwada, 1939, p.233). None has reported the paranemic type characteristic of first anaphase sister chromatid spirals. The tertiary split has been observed at various stages during meiosis (Table I) from diakinesis on, and is even considered by Stebbins (1935) to occur in the premeiotic anaphase or telophase chromosomes. A further "quarternary split" has been reported by Nebel (1935), Nebel and Ruttle (1936), Goodspeed, Uber and Avery (1935), Ruttle and Nebel (1937), and Hughes-Schrader (1940).

Table I

Observations of Tertiary Splits in the Meiotic Major Spiral

Organism	Stages	Form of spiralled half-chromatids	Reference
Tradescantia	Anaphase I	?	Nebel, 1932
"	Anaphase II	plect.	Geitler, 1935
"	Interkinesis	?	Kato, 1935
"	Anaphase II	?	Sax & Sax, 1935
"	Division II	?	Kuwada, 1938
" & Rhoeo	Anaphase I	?	Kaufmann, 1931
Rhoeo	" II	?	Sax, 1935
Gasteria	Anaphase II	plect.	Taylor, 1931
Galtonia	Anaphase II	plect.	Smith, 1932
Scilla	Division II	intertwined	Hoare, 1934
Allium) Tulipa) Paeonia)	Premeiotic A,T	?	Stebbins, 1935
Sagittaria	Interkin.,Div.II	?	Shinke, 1934
Hyacinthus	Anaphase II	?	Naithani, 1937
Lilium	AI-interkinesis	plect.	Chandler et al, 1937
Aloë, Allium	Anaphase II	diagram. plect.	Koshy, 1937, 1933
Zea	Diakinesis	?	McClintock, 1938
Trillium erectum	Met.I to Tel.II	?	Huskins & Smith, 1935
" "	Anaphase II	plect.	Wilson & Huskins, 1939
" "	Div.I (asynaptic)	plect.	Hunter (unpub.)
T.kamtschaticum	Met.I & Anaph.I	?	Shimakura, 1937
T. ovatum	Metaphase I	?	Warmke, 1937
T. Smallii	Diakinesis—on	?	Iwata, 1940
Dissosteira	Anaphase II	?	Nebel & Ruttle, 1937
Podisma	Anaphase I	?	Makino, 1936

Structure of Somatic Chromosomes.

While somatic chromosomes have been very extensively studied by cytologists for over forty years, a clear understanding of their spiral structure has lagged considerably behind the elucidation of meiotic chromosome structure. There is no dearth in the early literature of descriptions or illustrations of telophase and prophase spirals, but the relatively rare observations of metaphase or anaphase spirals led to the conclusion that the appearance of spiral structure was the exception rather than the rule (e.g., Mohr, 1914). It is now apparent that one of the most important factors responsible for this unfortunate situation is the fact that techniques which successfully demonstrate spiral structure in meiotic chromosomes usually show relatively little in the way of somatic spirals at metaphase and anaphase. The greater chromaticity of the matrix, as well as the close packing of the gyres of somatic chromosomes are presumably involved in determining this characteristic difference. Much confusion has also arisen from observations of chromosomes showing a highly vacuolated structure, which is now generally considered to be an artifact resulting from poor fixation (Darlington and La Cour, 1938; Geitler, 1938). In spite of these obstacles, the evidence from the many recent papers demonstrating somatic metaphase and anaphase spirals (Table II) is now sufficient to have changed the

Table II

Spiral structure of somatic chromosomes at metaphase and anaphase.

Organism	Stages	Form of spiral	Reference
Tradescantia	M,A,microspore	single	Sax and Sax, 1935
"	A, "	"	Geitler, 1935
"	M, anther wall	"	Patau, 1939
"	A, root tip	plect.	Kaufmann, 1926
"	A, stamen hair	"	Telezynski , 1930
"	A, root tip	?	Robyns, 1924
Galtonia	M,A, root tip	plect.	Smith, 1932
Gasteria	M, microspore	single	Geitler, 1935
"	M, "	"	Hillary,'40(Coleman)
Allium	A, root tip	"	Belar, 1926
"	M, microspore	"	Geitler, 1935
Tulipa	M,A, pollen tube	"	Upcott, 1936
"	M, microspore	"	Darlington & Upcott,'41
"	M, premeiotic	"	Coleman, 1940
Paris	A, integ. & nucellus	"	Geitler, 1938
"	M,A, root tip	"	Darl. & LaCour, 1938
Lilium	pollen tube	?	Sax (unpub.)
"	M,A, root tip	plect.(open)	Hsu-Siang, 1932
Haemanthus	A, root tip	"	Telezynski, 1931
Vicia	A, root tip	single	Kuwada, 1926
T.grandiflorum	M, root tip	"	Coleman, 1940
T.kamtschaticum	M, microspore	?	Matsuura, 1937c
Aloe & Adoxa	M, microspore	single	Geitler, 1935
Narcissus	M, microspore	"	Geitler, 1935
Scilla	pollen tube	?	Hillary, 1940
Osmunda	A, sporangial	?	Manton, 1939
Spirotrichonympha	mitosis	paranemic(?)	Cleveland, 1938
Ascaris	M, mitosis	?	Vejdovsky, 1912

problem from that of proving the existence of the spiral to one of determining its structure.

In addition to those listed in Table II as plectonemic, somatic anaphase chromosomes have been described as being composed of two intertwining threads in quite a variety of plants and a few animals: Tradescantia (Kaufmann, 1926; Husted, 1936); Podophyllum (Kaufmann, 1926, also diagrammatically, 1936); Trillium (Sharp, 1929; Warmke, 1937; Jeffrey, 1937; Mensinkai, 1939); Narcissus (Hedayatullah, 1931); Lilium (Hsu-Siang, 1932); Galanthus (Perry, 1932); Allium (Koshy, 1933; Mensinkai, 1939); Scilla (Hoare, 1934); Aloe (Koshy, 1937); Hyacinthus (Naithani, 1937); Pallavicinia (Wolcott, 1937); Osmunda (Manton, 1939); Amphibia (Schneider, 1910; Dehorne, 1911; Creighton, 1938); Tryxalis (telophase) (Brunelli, 1910); Collozoum (Patau, 1937).

Nebel and Ruttle (1936), Kuwada (1939) and Kuwada and Nakamura (1940) consider that the somatic anaphase chromosome contains independently spiralized chromatids which may be more or less relationally twisted, but less extensively intertwined, than those considered above. Non-intertwining threads in somatic anaphase chromosomes are described by Sharp (1929) in Vicia and diagrammatically by Straub (1938).

Abraham (1939) in Lilium finds two free parallel spirals at early anaphase which, subsequent to anaphase separation, become progressively twisted about each other.

At telophase the two chromonemata become so closely associated that the split is no longer apparent.

As indicated in Table II anaphase somatic chromosomes are considered by many to contain a single spiral (also by Belar, 1929; Belling, 1933; Gustafsson, 1936; Darlington, 1937, p.33; Mather, 1937; White, 1937; Afify, 1938) rather than two loosely interwoven chromonemata. However, in some cases (Smith, 1932, 1934; Geitler, 1935, 1938; Sax and Sax, 1935; Mensinkai, 1939; Coleman, 1940 a and b) it is considered that an apparently single spiral may actually be composed of two or more closely appressed chromonemata, which may not appear visibly distinct until telophase or the following prophase. The rather contradictory views in the literature on the number of chromonemata Kuwada (1939) explains "by the assumption that under certain circumstances the four chromonemata appear by union to be two or even one".

It is apparent that there is considerable disagreement in the literature regarding the number of chromatic threads visible at different stages. However, since the appearance of the split varies within single cells and even chromosomes, it is not surprising that the results of investigators using different techniques on a variety of organisms are not in complete accord. It seems not unlikely that environmental (e.g. moisture, Gustafsson, 1936) or mechanical factors (e.g. stretching) or normal physiological changes may reunite, optically at least, previously clearly separated chromonemata.

Relational coiling.

The twisting about each other of sister chromatids during late prophase and early metaphase has been figured and described in the literature for many years (Overton, 1905; Bonnevie, 1908; Fraser and Snell, 1911; Lee, 1924; Belar, 1926, 1929; Sharp, 1929; Taylor, 1931; Perry, 1932; O'Mara, 1933, Huskins and Hunter, 1935; Sax and Sax, 1935; Husted, 1936, 1937; Sax, 1936; Kaufmann, 1937; Warmke, 1937; Wolcott, 1937; Creighton, 1938; Flory, 1938; Shmargon, 1938; Csik and Koller, 1939; Levan, 1939; Manton, 1939; Eigsti, 1940). Only comparatively recently, however, has it begun to receive the attention which might be expected to be paid to such a characteristic property of prophase chromosomes and it is only during the last five or six years that any very commendable attempts have been made to relate relational coiling to a general scheme of chromosome behaviour.

It is apparent that if prophase chromatids are twisted about each other they must either be twisted in the same direction as the relic coils or in the opposite direction. A considerable number of cases of the former have been reported (Table III), but relatively few of the latter, (Darlington, 1937; Upcott, 1938).

Various opinions regarding the origin of chromatid relational coiling have been expressed in the literature.

Table III

Observations of plectonemic spirals (mostly relic coils)

Organism	Stage	Reference
Fritillaria aurea	microspore prophase relics	Darlington, 1935 p.46
" Elwesii	" " "	" , 1936 p.251
Hyacinthus	" " "	Upcott, 1938
Trillium kamtschaticum	" " "	Matsuura, 1938
Trillium erectum	second telophase (fig.7)	Huskins & Smith, 1935
" "	major coil	Hunter (unpub.)
" "	" "	Wilson & Huskins, 1939
Tradescantia	" "	Darlington, 1937, p.488
"	somatic prophase relics	Kaufmann, 1926
Vicia	" " "	Robyns, 1924
Aloë	" "	Koshy, 1937
Nothoscordum	" " relics	Koerperich, 1930
Haemanthus	" " (diagrammatic)	Telezynski, 1931
Orthoptera	spermatogonial prophase relics	White, 1940
Not given	chromosome model	Heitz, 1935

Some authors consider that chromatid relational coiling seen at late prophase is the result of an active process of twisting during earlier prophase stages (Darlington, 1937; Upcott, 1938; Csik and Koller, 1939; Kuwada, 1939). Husted (1936) and Abraham (1939) consider that it is carried over from the previous division, a relatively small amount being present at early anaphase, but considerably more at later stages. (Other evidence in the literature for double intertwining or interlocking anaphase chromatids has already been mentioned p.20). In contrast to those authors who consider that relational coiling is an active process, several investigators attribute relational coiling to the plane of cleavage being such that the resultant half-chromatids, or chromonemata, are relationally coiled at their inception (Kaufmann, 1926a; Koshy, 1937; Naithani, 1937; Kuwada, 1939).

Following this general introduction to the literature on relational coiling, we shall now consider the more important papers on the subject in somewhat greater detail.

Darlington's theory of crossing over is based on the assumption that relational coiling is associated with a torsion, which eventually becomes strong enough to break the chromatids. The broken ends can then rejoin in such a way as to form a chiasma. Apparently, on the assumption that somatic prophase chromatid relational coiling and meiotic prophase chromosome relational coiling are related, Husted (1938) and Csik and Koller (1939) have compared somatic

chromatid relational coiling with chiasma frequencies. Their data are not conclusive, but merely indicate a possible correlation between the number of relational twists per unit length of chromosome and the chiasma frequency.

Sax (1936) has studied relational coiling in somatic prophases of T. grandiflorum and Vicia Faba as an indirect method of analysing the "minor coil". He apparently considers that the minor coil determines the relational coiling either as a result of uncoiling the relics or by the development of the new prophase spirals within each chromatid.

Darlington (1936) and Upcott (1938) both seem to consider that two kinds of chromatid relational coiling occur in mitosis, but fail to give any criterion for adequately distinguishing them. The split, which, according to Darlington, does not occur until after the resting stage, gives rise at early prophase to a relational coiling which disappears along with the relic coils. The metaphase relational coiling is "due to the chromatids being dragged round one another during spiralization....." by a strain imposed on the chromatids by their developing internal spirals. Husted (1936) also attributes prophase relational coiling to the formation of new internal spirals, as are Upcott's "true" relational coils (see below). Since

Darlington considers the internal spiral to be the visible counterpart of the molecular spiral, the relational coil should be "as consistent and characteristic in direction as the molecular spiral", i.e., consistent within an arm (Darlington, 1935).

From a study of relic and relational coiling in Hyacinthus microspores Upcott (1938) describes two types of relational coiling which are essentially the same as Darlington's. The "true" relational coiling is generally consistent within an arm, but the other type of coiling which has the appearance of relational coiling, but, by inference, is not, compensates for the relics and is in the opposite direction. She finds no consistency in direction between "true" relational and relic coiling and concludes that this implies that direction of coiling at one division has an indeterminate relationship with that of the next.

Koshy (1937) suggests that relational coiling in anaphase and prophase mitotic chromosomes of Aloë results from what he terms a "spiral cleavage", occuring prior to metaphase. Contraction during prophase accompanies the formation of a new secondary spiral within each of the relationally coiled chromatids. The direction of relational twisting reverses at the attachment region in all chromosomes examined. Unwinding which proceeds from the ends towards

the attachment constriction is effected by the two arms rotating in the same direction after the chromosome becomes fixed to the equatorial plate at metaphase (Koshy, 1933).

Naithani (1937) also finds from his study of somatic chromosomes of Hyacinthus root tips that the "line of cleavage is along the longitudinal axis of the spiral chromonema thread". The gradual reduction of relational coiling during prophase is explained as follows:

> "With the commencement of the prophase the chromonema threads begin to undergo new spiralling, which gradually shortens and thickens the chromosomes. Each chromonema thread coils in a direction opposite to that of its partner and away from each other. The unravelling of the two chromatids may thus be explained as due to this mode of opposite coiling of the chromonema threads. If, as suggested, this new coiling is in opposite directions in each chromonema, then the two chromatids will go on unravelling until they come to lie side by side, the one being the mirror image of the other, giving a symmetrical pattern".(p.142)

In contrast to Koshy, who considered that unwinding occured at metaphase, Naithani found that it was completed before metaphase in the small chromosomes and during metaphase in the long ones.

Abraham (1939) finds that early anaphase somatic chromosomes of Lilium have two independently spiralized chromonemata which develop relational twisting by late anaphase. The split is obscured during telophase, but

when the duality reappears at prophase a further increase in relational coiling has taken place as previously reported by Husted (1936). Abraham states that "differential contraction might be responsible for the increase in twisting up to telophase" (p. 559), but that further increase apparent at prophase is presumed to be due to inter-chromatid adjustments induced by chromosome elongation in a limited space at telophase" (p.565), which he suggests may be accompanied by the ends of the chromosomes rotating in opposite directions. During prophase the twists are eliminated by a reversal of the telophase changes responsible for their formation. Each prophase chromatid divides to give rise to two free and independent spirals. Of the two possible methods of spiralization which would give rise to what he describes as "balanced" and "unbalanced" spirals, he chooses the former "in which the twisting caused by spiralization is compensated by a reversed internal twisting at every gyre of the spiral". The unbalanced type is comparable to our plectonemic and would give rise to intertwining half-chromatid spirals. Since these were not observed, he argues in favour of the balanced spiral which allows free separation of the daughter spirals.

Mensinkai (1939) regards somatic anaphase chromosomes of Trillium and Allium as composed of two intertwined chromatids. At telophase they tend to become closely appressed and appear as a single spiral until prophase is well advanced.

During prophase sister chromatids shorten by developing new internal coils which release the relational coils by the chromatids slipping around each other. He considers that "the slipping process always starts at both ends of the chromatids and proceeds toward the centromere, though the contraction itself and new spiral development might well have started all over the chromosome arms simultaneously". In direct contrast to Abraham's theory concerning coiling, Mensinkai states that "spiralization by the rotation of chromosome ends is excluded". He considers that it "seems to be caused by pH changes in the nucleoproteins of the chromatin".

Manton, (1939) describes two types of spirals "torsional" and "non-torsional", which are comparable to our plectonemic and paranemic. Further, she distinguishes between "constructional" spirals, which are perfectly stable in that they have no tendency to uncoil, and a second type which "are inherently unstable since the contorted object will tend to return to its original shape when the stress is removed". She regards chromosome spirals as "constructional" and considers that no torsional strains, i.e., tendency to uncoil, are present except at prophase. The problem as she sees it is not why should chromosomes be constructionally coiled, but why do the old spirals untwist during mitotic prophase as the new spirals are forming. "The only probable explanation of this would

appear to be a change in internal symmetry in each chromatid", which is only another way of describing Darlington's molecular reorientation.

Changes of internal symmetry have also been considered by Cooper (1938) and later by Barber (1940). The former suggested that a synapsing chromosome is asymmetrical in that it possesses but one limited pairing surface, and Barber implies that in mitosis chromatids would have the same specificity. Independent coiling of the chromatids at prophase "will convert the dorsiventral symmetry of the chromosome thread into a radial symmetry. Any remaining attraction will presumably be equal in magnitude all round the chromosome. That this must be so is proved by the gradual disappearance of relational chromatid coiling at mitosis during prophase (Upcott, 1937)".

Two recent papers by Japanese workers have considered the various possible arrangements of chromonema and chromatid spirals. Kuwada (1939) concludes "that when it is assumed that four chromonemata are contained in a single (anaphase) chromosome, and that the spirals which they form are orthospirals, different opinions on many problems can be harmonized". Somatic prophase chromatids thus would each contain two half-chromatids which at an early stage are in the form of an orthospiral, but untwist as they spiralize, so that by anaphase they may be completely untwisted and

independently spiralled, or they may have a few relational twists, or they may even form a "double-stranded spiral". Apparently Kuwada considers that untwisting of orthospirals takes place one division in advance of that at which they separate to opposite poles, and that prophase chromatid relational coiling is a secondary relationship, for he states "in the untwisting of the half-chromatids in the prophase, its two chromatids containing them will become intertwined with each other if in these chromatids the half-chromatid spirals, or the old spirals are of the same direction" (p. 245). Kuwada and Nakamura (1940), while apparently unable to choose between two and four chromonemata per (anaphase) chromosome, seem to agree with Kuwada's (1939) earlier paper in favoring a four stranded structure composed of two more or less independent double-stranded spirals.

Direction of coiling.

That the direction of coiling in chromosome spirals is not always consistent throughout has been noted by many investigators of spiral structure. Chromosomes whose gyres are neither all right-handed nor all left-handed may have reversals at the attachment region (interbrachial) or at regions within the arm (intrabrachial). As indicated in Table IV, both types of changes of direction have been found to occur in major, relic and relational coils in a variety of organisms.

Accurate determination of the direction of coiling in the minor spirals is more difficult, but Iwata (1935) states that the minor is usually coiled in the same direction as the major, but may sometime be reversed. Sax (1936), also states that "the direction of coiling of the major spirals is not necessarily dependent upon the direction of the minor spirals". However, Darlington (1935) postulates that both major and minor coils are in the opposite direction to the "molecular spiral", which is consistent within an arm.

Compared to meiotic stages relatively little is known about the direction of coiling in somatic spirals. However, changes in direction in prophase relic and relational coils, (Table IV) indicate that changes do occur in somatic chromosomes. Nebel (1939b) estimates "not more than one to occur per five gyres" in somatic chromosomes of Tradescantia.

Table IV

Changes in direction of coiling.

Organism	Stage	Type of changes observed	Reference
Secale	major coil	both	Sax, 1930
Gasteria	" "	intrab.	Taun, 1931
"	" "	both	Taylor, 1931
Tradescantia	" "	"	Nebel, 1932b
"	" "	"	Sax & Humphrey, 1934
"	" "	"	Nebel & Ruttle, 1935
"	" "	"	Darlington,'37 p.487
Sagittaria	" "	"	Shinke, 1934
Fritillaria	" "	interb.	Darlington, 1935
Lilium	" "	both	Iwata, 1935
Rhoeo	" "	"	Sax, 1935
Vicia	" "	"	Sax, 1936
Trillium	" "	"	Matsuura, 1935
"	" "	"	Huskins & Smith, 1935
"	" "	"	Matsuura, 1937a,b.
"	" "	"	Darlington, 1937 p.487
"	" "	"	Hunter, 1937
"	" "	"	Huskins, 1937
"	" "	"	Huskins & Wilson, 1938
"	" "	"	Wilson, 1939
"	" "	"	Wilson & Huskins, 1939
"	" "	"	Wilson & Hutcheson,'41
Veltheimia	" "	"	Coleman, 1940b

Table IV (continued)

Changes in direction of coiling. (continued)

Organism	Stage	Type of changes observed	Reference
Gasteria	microspore relic	intrab.	Taun, 1931
Paris	" "	interb.	Husted, 1937
Hyacinthus	" "	both	Upcott, 1938
Allium	resting nucleus	?	Cohen, 1937
"	root tip relics	?	Bonnevie, 1908
Ascaris	somatic relics	?	Bonnevie, 1908
Orthoptera	spermatogonial relics	both	Mohr, 1914
"	" "	"	White, 1940
Lilium	root tip rel. coils	?	Hsu-Siang, 1932
Allium	" " " "	interb.	Koshy, 1933
Nomocharis	" " " "	both	Darlington, 1936
Vicia	" " " "	"	Sax, 1936
Trillium	" " " "	"	Sax, 1936
Aloë	" " " "	interb.	Koshy, 1937
Hyacinthus	" " " "	"	Naithani, 1937
Trillium	microspore "	both	Huskins & Hunter, '37
"	" " "	"	Wilson, 1939
"	" " "	"	Wilson & Huskins, '39
Hyacinthus	" " "	"	Upcott, 1938
Tradescantia	" " "	"	Husted, 1936
"	somatic spiral	intrab.	Nebel, 1939b
Spirotrichonympha	" "	"	Cleveland, 1938

As to the consistency of coiling in chromosomes or parts of chromosomes, there has been considerable controversy. Direction of coiling within a chromosome has been reported to be non-random by Nebel (1932c), Koshy (1933), Sax and Humphrey (1934), Koller (1935), Sax (1935), Darlington (1935, 1936, 1937), Husted (1938), Upcott (1938) and Abraham (1939), whereas Matsuura (1935, 1937a and b), Iwata (1935), Hunter (1937), Huskins and Wilson (1938) and Wilson (1939) report direction of coiling to be random.

Hunter (1937) suggested from his study of coiling in synaptic, and asynaptic Trillium that "the factor which determines correlation in direction of coiling of both chromatids and chromosomes is closeness of association". Thus, in normal synaptic material sister chromatids coil in the same direction, but in asynaptic (type 2) they coil independently. Sister chromatids are also considered to coil in the same direction by Kuwada and Nakamura (1933), Sax (1936) and Matsuura (1937a); Shinke (1934) finds they generally coil in the same direction and Iwata (1935) finds no regularity at all in the direction of coiling of sister chromatids. These apparent discrepancies may be due to different degrees of closeness of the sister chromatids at the time of coiling, as suggested by Hunter and later by Wilson and Huskins (1939).

Direction of coiling in homologous chromosomes is considered to be random by Sax and Humphrey (1934), Matsuura (1935, 1937a), Sax (1936), Hunter (1937), Huskins and Wilson (1938) and Wilson (1939). Constant coiling patterns for homologous arms or chromosomes have been reported by Koller (1935), Darlington (1936, 1937) and Upcott (1938). Koshy (1933) even goes a step further, not only stating that "the twisting of the chromonemata is in opposite direction in the two arms of the chromosome", but that "the point at which reversal takes place appears to be determined by genetical factors". This, of course, would be possible only when the point, or points, of reversal are constant.

In addition to the attachment region, which seems to affect direction of coiling, chiasmata are also considered to interfere with coiling by Sax and Humphrey (1934), Huskins and Smith (1935), Nebel and Ruttle (1935), Sax (1936), Huskins and Wilson (1938) and Swanson (1941). Matsuura (1937b), however, considers that all intrabrachial changes of direction are fortuitous and that "the chiasma bear no primary relation to the occurrence of changes". The number of changes he considers to be proportional to chromosome length. Huskins and Wilson divide changes of direction into three distinct groups: (1) changes at the attachment, (2) changes due to chiasmata, and (3) changes not attributable to chiasmata nor to attachments, and whose numbers seem to be proportional to the number

of gyres. According to their hypothesis, direction of coiling is random both at chiasmata and at the attachment.

While a certain proportion of changes in direction are associated with attachments or chiasmata, not all changes can be attributed to these factors. Intrabrachial changes of direction in somatic spirals, in asynaptic chromosomes and those between non-terminalizing chiasmata obviously must fall into a different causal category. So far the only plausible explanation for them is that coiling may start simultaneously in more than one region, and if two regions coiling in opposite directions meet, a change will result (Wilson and Huskins, 1939).

The role of changes of direction in the mechanics of spiralization remains obscure, but at least their discovery has made a fundamental contribution to cytology by rendering invalid certain hypotheses of coiling which require a uniform torsion, twisting or rotation, and hence do not allow for intrabrachial changes of direction (Catcheside, 1932; Koshy, 1933; Darlington, 1935; Abraham, 1939; MacKnight, 1940; Coleman and Hillary, 1941). Other possible roles of changes of direction in the mechanics of spiralization will be considered in the discussion.

MATERIALS AND METHODS.

Rhizomes of Trillium erectum were collected near Ste. Agathe, Que., in September or October of each year, at which time their pollen mother cells are in pre-meiotic stages--they normally undergo meiosis in the very early spring. T. grandiflorum rhizomes, which ordinarily undergo meiosis during September, were collected in August on Ile Perrot, Que. If obtained in premeiotic stages, the time of onset of meiosis can be controlled to a considerable degree by temperature, but cannot be delayed indefinitely. The rhizomes brought to the laboratory were kept under various temperatures and conditions until used (see Table V)--some undergoing meiosis at temperatures as low as 2-3°C.

Material was prepared according to the 2BD-crystal violet aceto-carmine and Feulgen techniques. The 2BD-crystal violet schedule, essentially as employed by Huskins and Smith (1935), gave excellent spiral structure in T. erectum, but comparatively poor results in T. grandiflorum pollen mother cells. For relational coiling the best preparations of various stages during the first division of the microspore were obtained by smearing fresh anthers, then fixing with 3:1 alcohol-acetic fluid in a partial vaccum for three to seven minutes, replacing the fixative with 45 per cent acetic acid and staining with iron aceto-carmine. However, clearer spiral structure was obtained in microspore chromosomes using iron-aceto-carmine without the pre-fixation in alcohol-acetic.

After staining, the aceto-carmine was replaced with 45 per cent acetic acid and the sealed slides kept in a refrigerator except when being used. Warmke's (1935) alcohol--HCl method was used successfully to remove microspore walls where this was deemed necessary to give better definition and allow more flattening out of the nucleus. Root tips were fixed in 3:1 alcohol-acetic at 60°C and stained by the Feulgen method. All the root tip preparations were made permanent by a modified McClintock method, but most of the microspore slides were left in 45 per cent acetic acid.

Pollen was germinated and grown in hanging drops of 3 and 5 per cent sugar solutions and tested at various intervals varying from 12 to 60 hours. Pollen tube mitoses are most numerous between 30 to 50 hours, but there is considerable variation between slides. Aceto-carmine stain was used either directly or after fixation in 3:1 alcohol-acetic. Excellent results were obtained by fixing with alcohol-acetic in a partial vacuum as described above, and staining with iron aceto-carmine.

Observations were made with Zeiss 1.5 mm., 1.3 N.A. and 3 mm., 1.4 N.A. objectives combined with 7x, 15x and 20x oculars. All drawings used for measurements were made with the camera-lucide at original magnifications of 4000x and 3700x. Measurements were made from these drawings and carefully

checked. All lengths given are totals for a complement of 5 chromosomes unless otherwise stated. For the most part photomicrographs were taken with a 7x ocular and a 3mm., 1.4 N.A. objective.

The following formula has been used in calculating lengths of coiled chromonemata:

$$L = n \sqrt{p^2 + (\pi d)^2}$$

where L = the length of the chromonema, n = the number of gyres, p = pitch, and d = the gyre diameter measured as the outside diameter of the coil minus the diameter of the chromonema. Both diameters were the means of not less than five measurements per cell. The magnification of erros which may arise through the use of this formula was pointed out by Wilson and Huskins (1939) and one-plane measurements were favoured. In the present study particularly careful checking of the diameter of the major coil and of the chromonema itself has been carried out to minimize this effect. While we have given lengths to the nearest micron (except means), it should be emphasized that we do not consider our measurements to have an experimental error within such small limits. We agree with Manton (1939) that exact determination of chromosome length is extremely difficult, and consequently regard our data, not as precise measurements, but rather as close approximations.

When used as relative lengths they enable us to reach certain conclusions concerning the rhythmic changes in the spiralizati cycle. It is these changes with which we are concerned in the present paper, rather than with the exact determination of length per se.

As measurements were made from both aceto-carmine and 2BD-crystal violet preparations, it was deemed necessary to determine the relative degree to which these two fixatives alter the dimensions of the chromosome and chromonema. It is readily apparent that, relative to 2BD, acetic acid fixation swells both chromosomes and chromonemata. An estimate of the resultant difference in chromonema length has been obtained by comparing first anaphase lengths from 2BD-crystal violet and aceto-carmine preparations made from the same bud at the same time (Table XI). These give a conversion factor of 1.6. Since this factor is only an approximation, and may be inaccurate when extended to other stages, it should be pointed out that our main conclusions are not dependent upon the validity of the above factor, as we have enough data from the same fixative to draw most of our conclusions.

OBSERVATIONS

Length Changes

Data have been obtained on length changes during the chromosome spiralization cycle from early meiotic prophase, through first and second anaphases, at prophase, metaphase and anaphase of the following microspore division and at prophase of the pollen tube mitosis.

For the sake of clarity "chromosome length" has been used to designate distance along the chromosome, while "chromonema length" is applied to the spiralled or partially spiralled chromatic thread within the chromosome. At meiotic prophase stages, before the appearance of any visible coiling, chromonema and chromosome length are one and the same, but will be referred to as chromonema length. A further difficulty in terminology arises in referring to length changes between second anaphase and microspore prophase, due to the fact that during "resting stage" the chromonema undergoes a nomenclative change and emerges in the relic coil as a "chromatid". With the gradual loss of the relic coil during prophase, and the development of a new somatic spiral within each chromatid, we again have a "chromonema length", (the length of the spiralled thread) in contrast to a chromatid or chromosome length, the former, in the present discussion, being applied mostly to prophase, the latter more to metaphase and anaphase stages.

A résumé of chromosome and chromonema length changes at various stages of gametogenesis is given in Table V. Except for an apparent elongation between leptotene and zygotene, contraction occurs during meiotic prophase, reaching a maximum at early diakinesis. Chromonema elongation occurs between early diakinesis and first anaphase and between metaphase and anaphase of the microspore mitosis. Chromatid length at early microspore prophase is also considerably greater than meiotic anaphase chromonema length, but at later stages is much shorter. This chromatid contraction is presumably associated with the elimination of relic and relational coiling and the formation of a new somatic coil, whose gyres seem to increase in diameter and decrease in number during prophase.

Following this brief outline of length changes, we may now proceed to a detailed consideration of the various stages. Lengths at early meiotic stages were calculated from the illustrations of Huskins and Smith (1935). Pachytene lengths were measured from their drawings of whole chromosomes, while zygotene and leptotene lengths were estimated from the interchromomeric distances in sections of chromosomes relative to these distances at pachytene. These estimates show an increase from 920 μ at leptotene to 1040 μ at zygotene, followed by a decrease to 640 μ at pachytene. This contraction ratio of 16:10 agrees

Table V

Chromosome and Chromonema Lengths with Details of Material.

Stage	Chromatid or Chromosome A.C.	2BD	Chromonema A.C.	2BD	Material & Slide Number	Year Collected	Approx.temp. during meiosis	microspore mitosis
					Trillium erectum			
Leptotene	-	-	920	575	H+S(1935)Pl.I,fig.1			
Zygotene			1040	650	H+S(1935)Pl.I,fig.2			
Pachytene			640	400	H+S(1935)Text fig.3a,c,f			
Diplotene, late			186	116	H+S(1935)Text fig.2a			
Diakinesis,mid	104	65	160	100	H+S(1935)Pl.I,fig.8(corrected for chromosome A)			
early	86	54	109	68	58-4E-1 chrs.slightly coiled	1937	12°C	
early	106	66	133	83	58-5d-3 chrs.slightly coiled	1937	16°C	
mid	126	79	175	109	65-L2-a chrs.half-coiled	1938	20-22°C	
mid	125	78	187	117	66-T-81c chrs. half-coiled	1939	8-10°C	
Metaphase I	99	62	320	200	H+S(1935)Pl.III,figs.22,23			
Anaphase I	99	62	320	200	H+S(1935)Pl.II,fig.18			
	86	54	284	177	58-4E-1	1937	12°C	
	91	57	318	199	58-5d-3	1937	16°C	
	112	70	320	200	65-L2-a	1938	20-22°C	
	90	68	313	197	66-T-81 B and C	1939	8-10°C	
	86	54	368	230	66-T-72	1939	3-4°C	
	93	58	342	214	66-T-69A	1939	3-4°C	
	84	52	349	218	66-T-89A	1939	3-4°C	
Anaphase II	85	53	292	183	58-5d-3	1937	16°C	
	72	45	303	189	66-T-72	1939	3-4°C	3-4°C
	72	45	334	209	66-T-87 A+B	1939	3-4°C	
Microspore Prophase) Early	549	343	1000	625	66-T-117A	1939	4-7°C	13-14°C
) Later	452	282	-	-	66-T-72	1939	3-4°C	3--4°C
Metaphase	110	69	-	-	63-RC	1938	3°C	18-22°C
	79	49	-	-	66-T-72-SB	1939	3-4°C	3 - 4°C
Pollen tube pro.	516	322	-	-	66-T-128-A	1940	3-4°C	18-22°C
					Trillium grandiflorum			
Anaphase I	98	61	374	234	69-G-34	1939	3-4°C	
			394	246	69-G-110	1939	?	20-23°C
Microspore Prophase	467	292			69-G-100 and 104	1939	?	20-23°C
Metaphase(mean)	91	57	650	406	69-G-92,93,94,100,104	1939	?	20-23°C
Anaphase (mean)	95	59	977	610	69-G-92,100,104	1939	?	20-23°C
Additional Slides Used for Purposes other than Determination of Lengths.					65 M and P	1938	15-23°C	
					63 RCD	1938	3°C	18°C
					63 RCT	1938	3°C	15°C
					63 RCI	1938	3°C	6-8°C
					58-2E-1	1937	4°C	
					58-5H-3	1937	16°C	

Note; Italicised figures were calculated by use of the 2BD-aceto-carmine factor 1.6, based on measurements from 66-T-81-B and C (See Text p. 41)

remarkably well with the 15:10 ratio for corresponding stages found by Manton (1939) in Osmunda. By diakinesis the chromosome is reduced to one tenth of its zygotene length. This contraction, unlike that of somatic prophases, is not accompanied by any visible coiling within the chromosome.

The relationship of length changes between diakinesis and anaphase and the development of the major spiral have been discussed by Wilson and Huskins (1939). Further observations on 140 diakinesis and 80 first anaphase chromosomes from four different plants of T. erectum are presented in Table VI, (Figs. 1, 2 and 3). In each case the mean chromonema length increases from diakinesis to anaphase, while the mean chromosome length decreases slightly. When all the data are considered together the mean difference between chromonema lengths at the two stages is about twenty times the standard error.

Coefficients of variability for diakinesis and first anaphase chromonema lengths have been calculated from all complements measured. The variability at diakinesis is significantly greater (four times the standard error) than at anaphase, despite the fact that no late diakinetic stages were measured. This indicates clearly that the chromonema length undergoes a greater change during diakinesis than at anaphase. Although chromonema length is more difficult to determine accurately in first metaphase chromosomes, the data so far accumulated

Table VI

Chromosome and Chromonema Lengths at Diakinesis and First Anaphase

(From material fixed in 2BD)

Material	Mean Chr. Length	Mean Ch'ma Length	Number Whole Sets	Total No. of Chromosomes A	B	C	D	E	Mean No. Gyres	Mean Diam.	Mean Chr. Length	Mean Ch'ma Length	Number Whole Sets*	Ratio Diak:Anaph. Lengths Chr.	Ch'ma
	Early Diakinesis										First Anaphase				
58-4E-1	54.3	67.6	5	6	6	6	5	6	43.2	1.25	53.5	177.0	4	0.98	2.62
58-5d-3	66.4	83.2	13	14	15	13	14	14	45.8	1.35	57.3	199.2	5	0.87	2.40
	Mid Diakinesis														
65-L-2a	78.8	109.0	5	5	5	5	5	5	59.2	1.0	69.5	200.0	2	0.88	1.83
66-T-81c	77.6	116.6	5	5	5	5	5	5	60.2	1.0	68.2	196.8	5	0.88	1.70

Mean chromonema length in 28 complete complements at diakinesis = 90.0u ± 3.6; v = 21.0 ± 2.9

Mean chromonema length in 16 complete complements at anaphase = 194.0u ± 4.4; v = 8.7 ± 1.6

* No odd chromosomes were analysed at anaphase as in diakinesis.

indicate that it is approximately the same as at anaphase. It is therefore reasonable to assume the correctness of the general impression gathered earlier, that it is chiefly during diakinesis that elongation occurring between diplotene and anaphase takes place.

A determination of the exact amount of elongation occurring between diakinesis and anaphase is hampered by two factors. The first is the fact that a minor waviness, apparent in anaphase chromonemata, has not been taken into account in calculating anaphase / diakinesis length ratios. The second difficulty is that, apart from errors of measurement, ratios determined from comparisons of any but the earliest and latest stages of elongation will obviously always be underestimates of the total elongation. Anaphase length being relatively stable, variations in the anaphase/diakinesis ratio will be upward to an extent dependent upon the stage of diakinesis measured, i.e., the earlier the stage, the higher the ratio. The ratio varies from 1.70 to 2.62 (Table VI), when determined from the mean diakinesis and anaphase lengths of each of the four plants examined. It ranged as high as 3.2 in measurements in individual pairs of diakinesis and anaphase complements from single plants. It seems reasonable to conclude, therefore, that chromonema elongation between earliest diakinesis and first anaphase is not less than threefold.

Mean chromosome lengths are shorter at anaphase than diakinesis in all four materials of Table VI. The ratios of anaphase to diakinesis length are not, however, significantly less than one, and chromosome contraction is, therefore, not statistically established. If there is a real change, it is obscured by the high variability in chromosome length in different cells (correlated in part with diameter). Further, at diakinesis opening-out between chiasmata reduces chromosome length if measured as the shortest distance from end to end, and efforts to take this factor into account by measuring along the chromatids failed to give significant results, since they introduced further variability. The present data, while failing to show a significant change in length, do not definitely rule out the possibility of a chromosome contraction between diakinesis and anaphase, but indicate that if such a contraction does occur it must be of very limited magnitude.

As there is normally no interkinetic resting stage in T. erectum or T. grandiflorum, the major coil of first anaphase persists relatively unchanged through second anaphase (Fig.4) with no marked change in the structure of the coil. However, when both stages are measured in cells from the same plant (Table VII, 66-T-72), gyre number, gyre diameter, chromonema and chromosome lengths and mean gyre length are all significantly less at second anaphase than at first,

Table VII

Measurements of first and second anaphase spirals and microspore prophase relic coils of 66-T-72 (T. erectum)

	Gyre Diam.	Chrom. Length	No. of Gyres	Ch'ma Length	Mean Gyre Length
Anaphase I	1.8	116	65	385	5.91
	1.8	82	63	365	5.80
	1.8	73	63	364	5.77
	1.8	76	62	360	5.81
	1.7	84	65	366	5.64
Total	8.9	431	318	1840	28.93
Mean	1.78±.020	86±7.7	63.6±0.6	368±4.5	5.79±.05
Anaphase II	1.5	86.0	64	314	4.92
	1.5	85.0	64	313	4.90
	1.4	65.0	65	293	4.51
	1.4	61.5	63	283	4.49
	1.5	71.5	62	301	4.85
	1.7	75.0	60	329	5.48
	1.5	65.0	60	290	4.84
	1.5	69.5	60	291	4.86
	1.6	73.0	61	315	5.16
	1.6	69.5	60	310	5.16
Total	15.2	721.0	619	3039	48.83
Mean	1.52±.029	72±2.57	61.9±0.6	304±4.3	4.88±.10

		Chtd. Length	
Microspore Prophase	60	497	8.28
	59	494	8.37
	59	488	8.27
	55	470	8.54
	57	468	8.21
	56	443	7.91
	56	433	7.73
	58	427	7.36
	59	401	6.96
	53	400	7.54
Total	572	4521	79.17
Mean	57.2±0.7	452±12	7.92±.16

showing that a slight contraction has occurred. Chromonema lengths were also measured at either first or second anaphase in three other rhizomes (Table VIII) which had been kept under the same conditions as 66-T-72. The data as a whole show the chromonemata to be shorter at second than at first anaphase, but when different plants are compared, the difference is not always statistically significant.

The matrix which envelopes the major coil disappears after the second division of meiosis and a new matrix, which follows the turns of the old major coil, develops around each of the former half-chromatids. The relic coils of microspore prophase (Figs. 10, 11, 12, 19, 27, and 31) persist to some degree until nearly metaphase (Figs. 14-17, 22, 23, 32-36), and our "chromatid lengths" (Tables IX and XIII) are measured along the gyres of one of the two relationally coiled constituent chromatids.

In addition to chromatid length the mean number of gyres and mean gyre length of the relic coil are given in Tables VII and IX for early prophase nuclei from two T. erectum rhizomes. Chromatid and mean gyre lengths in early prophase of the first microspore division are significantly greater than the corresponding measurements at either meiotic anaphase, even in comparisons between different plants. Differences in the mean number of gyres at meiosis and

Table VIII

Measurements of meiotic anaphase chromosomes from 66-T-69,-89 and-87 (T. erectum)

Plant No.	Gyre Diam.	Chrom. Length	No. of Gyres	Ch'ma Length	Mean Gyre Length
			Anaphase I		
66-T-69	1.6	86	64	333	5.21
	1.6	101	65	342	5.26
	1.8	92	63	368	5.84
	1.7	95	65	359	5.53
	1.4	91	67	308	4.61
Total	8.1	465	324	1710	26.45
Mean	1.62±.066	93±2.47	64.8±1.0	342±11	5.29±.21
66-T-89	1.8	98	63	369	5.86
	1.8	86	61	355	5.82
	1.7	77	63	345	5.48
	1.6	78	66	341	5.16
	1.6	81	65	336	5.15
Total	8.5	420	318	1746	27.47
Mean	1.7±.045	84±2.39	63.6±0.9	349±6	5.49±.16
			Anaphase II		
66-T-87	1.8	80	61	354	5.80
	1.6	76	60	311	5.18
	1.7	74	59	323	5.48
	1.8	72	58	336	5.79
	1.7	72	59	323	5.48
	1.8	75.5	61	352	5.78
	1.8	69	59	340	5.76
	1.7	64	60	326	5.44
	1.7	67.5	59	322	5.46
	1.9	68	58	352	6.08
Total	17.5	718	594	3339	56.25
Mean	1.75±.027	71.8±1.51	59.4±0.3	334±5	5.62±.09

Table IX

Chromatid lengths and gyre number in microspore prophases of 66-T-117 (T.erectum)

Cell	No. of Gyres	Chtd. Length	Mean Gyre Length
1	65	652	10.04
2	62	594	9.57
3	62	573	9.24
4	63	565	9.01
5	57	498	8.74
6	58	496	8.58
7	57	463	8.14
Total	424	3841	63.32
Mean	60.6 ± 1.2	549 ± 25	9.08 ± .24

microspore prophase are not always significant in comparisons of different plants, but in the single case in which gyre number was obtained at meiosis and at microspore prophase (66-T-72), there is a small but significant decrease. It is obvious that if an elongation occurs at the same time that gyres are being lost the mean gyre length must increase. This could be effected by an increase, either in pitch, or in gyre diameter, or a combination of both. Apparently the latter occurs in the transition of the major coil to the relic during the interval between second anaphase and microspore early prophase of T. erectum.

A summary of the means for all measurements made on T. erectum, and already presented in Tables VII to IX, is given in Table X. Measurements used in calculating the 2BD aceto-carmine conversion factor (see p.41) are given in Table XI.

Similar, but less extensive, measurements have also been made at first anaphase and microspore prophase in T. grandiflorum (Tables XII and XIII). The difference between anaphase chromonema and prophase chromatid lengths is again significant, although we are comparing anaphase lengths, which vary relatively little with prophase stages which are undergoing contraction and hence are highly variable. At a relatively early stage of microspore prophase the chromatid

Table X

Summary of means with their standard errors from data presented in tables VII, VIII, & IX (T. erectum)

Plant No.	No. of Cells	Gyre Diam.	Chrom. Length	No. of Gyres	Ch'ma or Chtd. Length	Mean Gyre Length
			First Anaphase			
66-T-72	5	1.78 ± .020	86 ± 7.70	63.6 ± 0.6	368 ± 4.5	5.79 ± .05
66-T-69	5	1.62 ± .066	93 ± 2.47	64.8 ± 1.0	342 ± 11.0	5.29 ± .21
66-T-89	5	1.70 ± .045	84 ± 2.39	63.6 ± 0.9	349 ± 6.0	5.49 ± .16
-72,69,89	15	1.70 ± .031	90 ± 3.00	64.0 ± 0.4	353 ± 4.8	5.59 ± .07
			Second Anaphase			
66-T-72	10	1.52 ± .029	72 ± 2.57	61.9 ± 0.6	304 ± 4.3	4.88 ± .10
66-T-87	10	1.75 ± .027	72 ± 1.51	59.4 ± 0.3	334 ± 4.7	5.62 ± .09
-72,-87	20	1.64 ± .033	72 ± 1.45	60.6 ± 0.4	319 ± 4.8	5.28 ± .16
			Microspore Early Prophase			
66-T-72	10			57.2 ± 0.7	452 ± 12	7.92 ± .16
66-T-117	7			60.6 ± 1.2	549 ± 25	9.08 ± .24

Table XI

First anaphase measurements used in calculating the 2BD-aceto-carmine factor.

Plant No.	Gyre Diam.	Chrom. Length	No. of Gyres	Ch'ma Length	Mean Gyre Length		
66-T-81-B (aceto-carm.)	1.50	93	67	326	4.87		
	1.55	85	62	310	5.00		
	1.60	85	61	316	5.18		
	1.57	96	67	336	5.01		
	1.36	89	71	315	4.44		
	1.34	85	66	287	4.35		
	1.50	80	66	319	4.83		
	1.40	82	65	300	4.62		
	1.43	91	65	302	4.64		
	1.42	112	67	320	4.77		
Total	14.67	898	657	3131	47.71		
Mean	1.47±.028	89.8±2.97	65.7±.88	313.1±4.5	4.77±2.62		
66-T-81-C(2BD)				2BD	a.c.*	2BD	a.c.*
	1.00	66	64	212	339	3.31	5.30
	1.10	68	62	210	336	3.39	5.42
	1.06	71	56	194	310	3.46	5.52
	0.98	63	57	172	275	3.01	4.82
	0.94	73	62	196	314	3.16	5.06
Total	5.08	341	301	984	1574	16.33	26.12
Mean	1.02±.029	68.2±1.79	60.2±1.55	197±7.2	315±11.5	3.26±.081	5.22±.013

* Aceto-carmine equivalents to the 2BD measurements, obtained by multiplying by the factor 1.6.

Table XII

Measurement of first anaphase chromosomes of 69-G-110 and 69-G-34* (T.grandiflorum)

	Gyre Diam.	Chrom. Length	No. of Gyres	Ch'ma Length	Mean Gyre Length
69-G-110	2.0	85	56	358	6.40
	1.9	71	63	383	6.08
	1.8	103	70	394	5.63
	2.2	82	64	426	6.67
	2.2	79	60	410	6.84
Total	10.1	420	313	1971	31.62
Mean	2.05±.082	84±5.29	62.6±1.6	394±12	632±.21
69-G-34*	1.0	55	69	224	3.24
	1.1	60	63	220	3.50
	1.2	66	66	256	3.88
	1.16	60	64	240	3.75
	1.1	63	65	232	3.57
Total	5.56	304	327	1172	17.94
Mean	1.11±.034	60.8±1.83	65.4±1.1	234±7	3.59±.11
Aceto-carmine equivalents					
Mean				375±11	5.74±.18
Mean of 69-G-110 & 69-G-34			64.0±0.5	385±8	6.0 ±.16

*Lengths for 69-G-34 are from 2BD-crystal violet preparations. Multiply by 1.6 to convert to aceto-carmine equivalents.

Table XIII

Chromatid lengths and gyre number in microspore prophases of T. grandiflorum

Plant No.	Cell	Stage	No.of Gyres	Chtd. Length	Mean Gyre Length
69-G-100	1	Early	68	591	8.69
100	2	Prophase	55	506	9.20
100	3		54	487	9.02
104	4		56	483	8.63
100	5		52	460	8.85
100	6		47	440	9.37
100	7		48	438	9.14
100	8		48	437	9.12
104	9		47	418	8.90
104	10		47	410	8.72
		Total	522	4670	89.64
		Mean	52.2±2.1	467±17	8.96±.08
69-G-104	1	Later	50	388	7.77
100	2	Prophase	46	378	8.22
104	3		45	376	8.36
104	4		54	372	7.45
104	5		41	362	8.43
104	6		47	346	7.36
		Total	281	2222	47.59
		Mean	47.5±1.5	370±8	7.93±.18

has again contracted to less than the meiotic anaphase chromonema length. The extent of the elongation after second anaphase will therefore be indicated only when the earliest prophase stages are measured and again almost all attempts to measure it will give underestimates.

Within the chromatids of early microspore prophase wavy or loosely-coiled chromonemata can often be seen in good preparations (Figs. 10, 11, 12, 13, 27, 28, 31). The length of these chromonemata is obviously greater than the chromatid length, but the extent of the difference is difficult to determine accurately. From measurements of short segments the chromonema length at early microspore prophase is estimated to be about 1000 μ for a chromatid length of about 550 μ. As the chromatids shorten the gyres of the developing internal spiral become more regular and more apparent (Figs. 32-36). By metaphase and anaphase the gyres are almost as regular as those of the meiotic major spiral, but more tightly packed (Figs. 37-42). Optical cross-sections of microspore anaphase chromosomes are shown in Figure 42. This is conclusive evidence that the somatic chromosomes contain a single spiral (but not necessarily a single-stranded spiral) and not two or more independently spiralized chromatids, as is frequently maintained (See p.20).

Gyre size and number and chromosome and chromonema lengths of the somatic spiral of microspore metaphase and anaphase chromosomes are given in Tables XIV and XV for five cells at each stage from each of three different plants. There are statistically significant differences between metaphase and anaphase mean gyre length, gyre diameter and chromonema length, both in individual plants and in the means for the fifteen cells from all three plants. Mean chromosome length and mean gyre number are also greater at anaphase but the difference is not significant. It is of interest that the mean chromonema length at anaphase is 977 ± 28.3, which is rather close to the estimated length at leptotene or zygotene. However, since no correction has been made for the apparent minor waviness of the anaphase somatic spiral the value given is probably an underestimate.

Counts of gyre number in the relic coils of pollen tube prophase nuclei (Table XVI, Figs.43-46) agree rather closely with the mean number of gyres in microspore anaphase chromosomes. However, mean gyre diameter, gyre length and chromatid length are all significantly less than corresponding values at the previous anaphase, indicating that considerable prophase contraction has already occurred in the nuclei examined.

Table XIV

Measurements of metaphase and anaphase somatic spirals in T. grandiflorum microspores.

	Gyre Diam.	Chrom. Length	No.of Gyres	Ch'ma Length	Mean Gyre Length
69-G-92-G Metaphase	1.80	107	101	520	5.12
	2.17	126	107	534	5.98
	2.47	81	114	610	6.95
	2.62	90	98	720	7.34
	2.65	98	98	738	7.48
Total	11.71	502	518	3122	32.87
Mean	2.34±.245	100.4±7.71	103.6±3.08	602.4±46.8	6.57±.45
Anaphase	3.44	103.5	95	935	9.90
	2.70	98.5	130	980	7.55
	3.03	95.0	126	995	8.90
	2.73	97.0	127	998	7.70
	3.62	111.0	110	1105	10.05
Total	15.52	505.0	588	5013	44.10
Mean	3.10±.185	101±2.87	117.6±6.64	1002.5±27.9	8.82±.53

Table XIV
continued

	Gyre Diam.	Chrom. Length	No. of Gyres	Ch'ma Length	Mean Gyre Length
69-G-100-G Metaphase	1.76	77.0	151	650	4.30
	2.03	83.5	120	655	5.49
	2.02	73.0	128	695	5.44
	1.59	100.0	172	700	4.06
	2.36	80.0	109	710	6.49
Total	9.76	413.5	680	3410	25.78
Mean	1.95±.131	82.7±4.7	136±11.3	682±6.1	5.16±.442
Anaphase	2.75	83	132	780	5.92
	2.37	71	132	836	6.34
	2.47	82	129	890	6.84
	2.44	95	148	1000	6.75
	2.67	112	146	1080	7.46
Total	12.70	443	687	4586	33.31
Mean	2.54±.072	88.6±6.98	137.4±4.0	917.2±27.3	6.66±.258

Table XIV
continued

	Gyre Diam.	Chrom. Length	No.of Gyres	Ch'ma Length	Mean Gyre Length
69-G-104-A Metaphase	1.73	70.0	126	570	4.52
	1.83	88.5	128	622	4.85
	2.05	109.0	119	662	5.55
	2.09	82.5	120	680	5.67
	2.20	92.5	114	685	6.01
Total	9.90	442.5	607	3219	26.60
Mean	1.98±.087	88.5±5.2	121±1.9	643.8±21.5	5.32±.275
Anaphase	2.48	97.5	133	920	6.91
	2.65	82.0	126	930	7.39
	2.50	97.5	138	958	6.95
	2.57	104.0	140	1015	7.25
	3.20	89.5	134	1230	9.19
Total	13.40	470.5	671	5053	37.66
Mean	2.68±.133	94.1±3.8	134±2.4	1010.6±39.9	7.5±.424

Table XV

Summary of means with their standard errors from data presented in table XIV (T.grandiflorum)

	No.of Cells	Stage	Gyre Diam.	Chrom. Length	No.of Gyres	Ch'ma Length	Mean Gyre Length
69-G-92-G	5	M	2.34±.245	100±7.7	104±3.1	603±46.8	6.57±.45
	5	A	3.10±.185	101±2.9	118±6.6	1003±27.9	8.82±.53
69-G-100-G	5	M	1.95±.131	83±4.7	136±11.3	682±6.1	5.16±.44
	5	A	2.54±.072	89±7.0	137±4.0	917±27.3	6.66±.26
69-G-104-A	5	M	1.98±.087	88±5.2	121±1.9	644±21.5	5.32±.27
	5	A	2.68±.133	94±3.8	134±2.4	1011±39.9	7.53±.42
69-G-92,100 and 104	15	M	1.95±.097	90.5±3.9	120±5.1	650±17.2	5.68±.27
	15	A	2.77±.099	95±2.9	130±3.3	977±28.3	7.67±.30

Table XVI

Measurements of relic coils at prophase of pollen tube division of T.erectum (66-T-128-A)

	Gyre Diam.	No.of Gyres	Mean Gyre Length	Total Length
	1.7	136	3.20	435
	1.8	116	4.00	464
	1.8	128	3.68	470
	1.8	132	3.59	475
	1.93	144	4.13	590
	2.3	132	5.00	660
Total	17.33	788	23.60	3094
Mean	1.89±.088	131±3.78	3.93±.080	516±33.9

Chromatid length and gyre number at various stages from early microspore prophase through metaphase and anaphase and up to prophase of the pollen tube mitosis are given in Table XVII. The data show that as chromatid length decreases during prophase contraction the gyre number decreases until metaphase is reached. Gyre numbers at metaphase, anaphase, and the following prophase do not differ significantly.

Relational Coiling.

The relic coils of early microspore prophase usually show their two chromatids optically separate for short distances only and these mostly at the ends (Fig.10, lower left). However, as prophase advances the doubleness becomes increasingly clear throughout (Figs. 13, 15, 20, 21, 32-35) and it is seen that the two chromatids of the relic coil form a plectonemic spiral (Text fig. 1). During early prophase contraction the gyres decrease in both size and number, and by mid to late prophase relatively few remain (Figs. 17, 22, 23, 32-35). While some unwinding by rotation may occur, it is obvious that for the most part gyres are being straightened out faster than their chromatids are untwisting. This gives rise to the relational twisting typical of late prophase (Figs. 15, 16, 22, 23, 26, 32-36, and Plate VIII). The relational coil thus formed is, of course, in the same direction as the relic from which it was

Table XVII

Chromatid length and gyre number during microspore mitosis and in pollen tube prophase nuclei.

Stage	Chromatid Length	No. of Gyres
Early microspore prophase (66-T-117)	565	about 700
Later prophase		
69-G-92-G	233	280
69-G-100-G	202	310
Prometaphase		
pollen tube (66-T-128-A)	181	270
Microspore (69-G-100-G)	100	172
Metaphase (Table XIV)		
(mean from 15 cells)	90.5±3.9	120±5.1
Anaphase (Table XIV)		
(mean from 15 cells)	95.0±2.9	130±3.3
Pollen tube prophase	-	131±3.78

derived. The earliest stage at which direction of the relational twists could be determined in all chromosomes was about mid-prophase or, more precisely, at a chromatid length of about 200-250 μ.

Fifty-four complete microspore complements of T. grandiflorum, at stages varying from mid-prophase to metaphase, have been analysed for chromatid length, number and direction of relational twists and mean intertwist distance. They were arranged in order of descending length and divided, for brevity in presentation, into four equal groups, totals and means for which are given in Table XVIII. Results for the E chromosomes are given separately so that comparisons can be made with root tip data in which only this, the longest chromosome, was studied. The steady reduction in number of twists as length decreases is obvious (Text fig.2). If we take length as an index of the stage of mitosis, then the amount of relational coiling is found to be highly variable at all stages measured. Individual microspores ranged in total chromatid length from 214 μ at mid-prophase to 63 μ at metaphase, and the number of twists from 48 to 0. Between the first and fourth group the mean length drops from 179.6±4.9 to 84.1±4.3 and the mean number of twists from 30.0±2.7 to 5.0±1.4.

In an earlier analysis, made primarily to determine direction of relational twists and therefore not including

Table XVIII

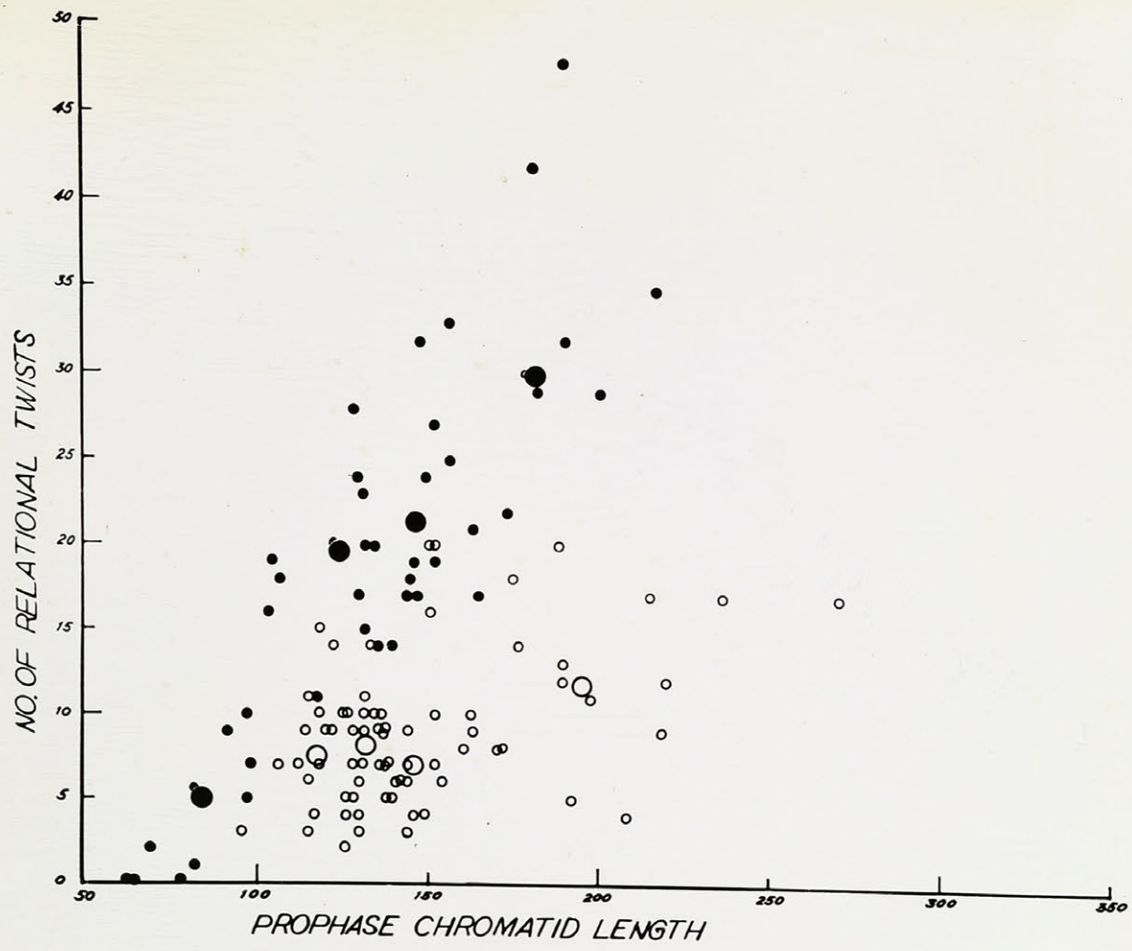
Analysis of chromatid lengths(L) and relational twisting in T.grandiflorum microspores from aceto-carmine preparations.

	Cell Nos.	Whole Complements L	No. of Twists	Changes Poss.*	Changes Obs.	L T+A**	E Chromosomes L	Twists	Changes	Direction of Twisting ***
Group 1	1	214	35	26	6	5.4	61	8	2	RRL . RRRRL
	2	198	29	20	6	5.8	57	9	3	LLRR . LLRRL
	3	187	48	39	7	3.5	51	14	3	RRRLL . LRRRRLLLL
	4	187	32	23	2	5.0	53	13	0	LLLLLLL . RRRRRR
	5	180	29	20	3	5.3	48	11	2	RRRR . LLLLRRL
	6	179	30	21	3	5.1	52	8	1	RRRLL . LLL
	7	178	42	33	5	3.8	48	14	2	RRRRLLL . RRRLLLL
	8	172	22	13	3	6.4	52	7	0	LL . LLLLL
	9	164	17	9	2	7.4	43	6	1	LLL . RRL
	10	162	21	12	5	6.2	41	4	0	L . RRR
	11	155	25	16	3	5.2	45	9	1	RRRL . LLLLL
sub-total		1976	330	232	45	59.1				
Group 2	12	154	33	24	5	4.0	46	10	1	RRRRR . LLLRR
	13	151	19	10	1	6.2	42	5	0	LLL . LL
	14	150	27	20	6	4.7	42	10	2	RRLRRR. . RRRR
	15	148	24	16	1	5.1	40	8	0	LLLLL . LLL
	16	146	32	23	6	4.0	44	10	2	LLRR . LLRRRR
	17	146	17	8	1	6.6	41	3	1	LR . L
	18	145	19	10	2	6.0	37	3	1	LR . L
	19	144	18	10	0	6.3	42	3	0	RR . L
	20	143	17	9	4	6.5	40	6	2	LRR . LLR
	21	139	14	7	2	7.3	40	5	0	RR . RRR
	22	135	14	7	2	7.1	40	4	1	RL . LL
sub-total		1601	234	144	30	63.8				
Group 3	23	133	20	12	5	5.3	42	7	3	LR . LRLLL
	24	131	20	12	0	5.2	38	8	0	LLLLL . RRR
	25	131	15	7	2	6.6	36	5	1	LL . LLR
	26	130	23	14	3	4.6	36	6	0	LLL . RRR
	27	129	24	16	6	4.5	38	9	3	RLLR . RRLLL
	28	129	17	9	2	5.9	39	7	1	RLL . RRR
	29	127	28	19	4	3.8	38	10	1	RRRRL . LLLLL
	30	122	20	12	2	4.9	32	5	0	L . LLLL
	31	117	11	4	2	7.3	40	2	0	R . L
	32	106	18	10	4	4.6	30	7	2	LRRR . RLL
	33	104	19	12	2	4.3	32	9	1	LLLLL . LLRR
sub-total		1359	215	127	32	57.0				
Group 4	34	103	16	9	0	4.9	31	5	0	L . RRRR
	35	98	7	0	0	8.2	27	2	0	L . R
	36	97	10	3	0	6.5	27	3	0	R . LL
	37	97	5	2	1	9.7	30	4	1	LL . LR
	38	91	9	3	0	6.5	26	4	0	RR . LL
	39	84	5	1	0	8.4	26	2	0	U . LL
	40	82	1	0	0	13.6	21	1	0	U . R
	41	78	0	0	0	15.6	22	0	0	U . U
	42	69	2	0	0	9.9	18	0	0	U . U
	43	64	0	0	0	12.8	17	0	0	U . U
	44	63	0	0	0	12.6	17	0	0	U . U
sub-total		926	55	18	1	108.7				
Total		5862	266	521	108		1668	266	38	

*the number of possible changes within an arm is one less than the number of twists.

** $\frac{L}{T+A}$ is mean intertwist distance, where L is chtd. length, T the number of twists, and A the number of attachments.

***U indicates that no relational twisting is present.



Text-figure 2.

Graph showing relationship between chromatid length and number of relational twists in microspores of T. grandiflorum (solid circles) and T. erectum (hollow circles). The large circles are means for the groups I - IV of Table XXII.

completely untwisted metaphase chromosomes, (Figs. 18, 38, 39) similar data had been obtained from 72 complete T. erectum microspores fixed in 2BD. Lengths have been converted into aceto-carmine equivalents and the data divided into four groups of 18 each (Table XIX). The mean lengths of these correspond roughly to the mean group lengths in T. grandiflorum, with the exception, of course, of the fourth group whose mean is greater in T. erectum, since it did not include the latest, untwisted metaphase stages. Individual microspores, in this species, ranged in total chromatid length from 269 μ at mid-prophase to 94 μ at the latest stage at which twists were still present. The number of twists ranged from 20 to 2. The mean length of the longest group was 194.1±6.8 and of the shortest 117.3±1.8. The mean number of twists in these two groups is 11.8±1.1 and 7.5±0.9.

From Table XVII and Text Fig. 2 it is evident that the relationship between prophase contraction and loss of twists is different in the microspores of the two species. At early prophase the mean gyre length (of the relic coil) has been found to be approximately the same in both species (Table X and XIII). The initial frequency of twists per unit length should therefore be approximately the same in both if our assumption that relational coiling derives from plectonemic coiling is correct. But at later prophase stages (Groups 1-3, Table XXII) it is apparent that chromosomes

Table XIX

Analyses of chromatid lengths and relational twisting in T. erectum microspores from 2BD-crystal-violet preparations.

	Whole Complements			E Chromosome			
Cell No.	Chtd. Length	No. of Twists	No. of Changes	Chtd. Length	No. of Twists	No. of Changes	Direction of Twisting
1	168	17	5	51	5	1	L.LLLR
2	147	17	3	45	3	1	L.LR
3	137	12	0	32	2	0	R.R
4	136	9	1	38	3	0	LL.R
5	134	17	4	40	4	1	LR.RR
6	130	4	0	38	1	0	U.R
7	123	11	1	27	2	0	L.L
8	120	5	0	31	1	0	U.L
9	118	13	2	34	3	2	U.LRL
10	118	12	1	36	3	1	LR.L
11	117	20	8	31	5	3	U.LRLRR
12	110	14	3	32	3	1	U.LRR
13	109	18	4	34	6	1	LLL.LRR
14	107	8	0	30	2	0	L.R
15	107	8	1	33	2	0	R.R
16	102	9	0	24	1	0	U.L
17	101	10	2	30	2	1	U.RL
18	100	8	1	26	3	0	L.RR
Subtotal	2184	212	36				
Mean	121.3±4.26	11.8±1.09					
19	96	6	0	27	2	0	R.L
20	95	10	1	22	4	1	R.LRR
21	95	7	0	26	2	0	L.L
22	94	16	3	26	5	0	LL.RRR
23	94	10	1	26	2	0	L.R
24	94	10	2	25	3	1	RL.R
25	93	4	0	26	2	0	R.R
26	91	4	0	24	1	0	R.U
27	90	9	0	23	4	0	RR.LL
28	90	7	0	25	2	0	L.R
29	90	6	0	23	1	0	U.L
30	90	3	0	26	2	0	L.L
31	88	6	0	25	2	0	R.L
32	88	6	1	28	3	1	L.RL
33	87	5	0	24	3	0	L.LL
34	86	7	1	24	2	1	U.RL
35	86	7	0	25	3	0	R.LL
36	86	5	0	22	2	0	L.R
Subtotal	1633	128	9				
Mean	90.7±.81	7.11±.72					

Table XIX (continued)

	Whole Complements			E Chromosome			
Cell No.	Chtd. Length	No. of Twists	No. of Changes	Chtd. Length	No. of Twists	No. of Changes	Direction of Twisting
37	85	10	0	22	2	0	LL.U
38	85	9	0	23	4	0	LL.RR
39	85	9	1	24	3	0	LL.L
40	85	9	0	21	3	0	L.RR
41	85	4	0	26	1	0	R.U
42	84	10	2	23	4	2	RL.LR
43	83	14	1	21	3	0	L.RR
44	82	11	0	21	1	0	U.L
45	82	10	2	20	4	2	RL.LR
46	82	9	0	21	3	0	U.LLL
47	82	7	0	25	1	0	U.R
48	81	6	0	22	2	0	L.L
49	81	4	0	22	2	0	L.R
50	81	3	0	20	1	0	U.L
51	80	9	1	23	2	0	R.R
52	80	7	0	20	2	0	L.R
53	80	5	1	22	1	0	U.L
54	79	10	1	20	3	0	L.LL
Subtotal	1482	146	9				
Mean	82.3±.49	8.11±.67					
55	79	5	1	22	2	1	RL.U
56	79	4	0	21	3	0	L.RR
57	79	2	0	22	1	0	R.U
58	78	10	0	20	2	0	L.L
59	76	14	0	24	5	0	RR.RRR
60	76	9	0	20	2	0	R.L
61	75	9	1	21	3	0	RR.R
62	74	15	1	23	4	1	L.LLR
63	74	10	1	18	1	0	U.L
64	74	7	0	22	2	0	L.R
65	73	4	1	20	1	0	R.U
66	72	11	1	23	4	0	RR.RR
67	72	6	1	20	2	0	LL.U
68	72	3	0	19	1	0	U.L
69	71	9	1	17	2	0	R.L
70	70	7	2	21	2	0	L.R
71	66	7	0	24	4	0	RR.RR
72	60	3	0	18	2	0	L.L
Subtotal	1320	135	10				
Mean	73.3±1.13	7.50±.88					

of T. erectum have far fewer twists per unit length than have those of T. grandiflorum. It seems probable that the timing relationship between contraction, i.e., length, and untwisting differs somewhat in the two species. If length is used as a criterion of the stage of mitosis, it appears that untwisting occurs earlier in T. erectum and proceeds more quickly at later stages in T. grandiflorum.

The mean intertwist distances for whole complements have been calculated (Tables XVIII and XXII) by dividing the chromatid length by the number of relational twists plus the number of attachments $\left(\frac{L}{(T + A)}\right)$. The means for comparable groups are seen to be consistently lower in T. grandiflorum than in T. erectum, a further indication that untwisting takes place earlier in the latter.

The E chromosomes from root tip cells of T. erectum and T. grandiflorum have been analysed for comparison with microspore chromosomes (Tables XX and XXI). Partly on account of the double number of chromosomes in root tips (Fig.26), it is much more difficult to obtain data on number and direction of twists from correspondingly early stages. There is consequently a much smaller range in chromatid length in the data presented. The range in number of relational twists, however, remains high and it is therefore difficult to determine a relationship between length and number of twists. Relic coils were observed in a large

Table XX

Chromatid length and relational twisting in fifty E chromosomes from root tip cells of T. grandiflorum.

Cell	Chtd. Length	No. of Twists	No. of Changes	Direction of Twisting
1	36	7	0	R*RR . RRRR
	38	6	0	RR*RR . RR
2	34	4	0	LL . LL
3	36	3	0	LL . L
	33	5	0	RRR . L
4	28	2	1	LR . U
	32	5	0	LL . RRR
5	38	5	0	L . RRRR
6	38	8	1	LLLL . RRLL
7	38	8	1	LLL*L . LRRR
8	51	16	3	L*LRRL*LL*LL . L*LRRRRR
9	36	5	0	LL . RRR
10	39	6	2	RLL . RRL
11	42	4	0	R . L*LL
	40	8	0	RRRR . RR*RR
12	40	3	0	L . RR
13	36	3	0	RR . L
	34	4	0	RRR . L
14	39	5	0	LL . LLL
	36	2	0	L . R
15	36	2	0	R . L
	35	3	0	LLL . U
16	36	6	1	RRR . RRL
	38	3	1	L . RL
17	34	1	0	L . U
	38	4	0	RR . LL
18	40	6	0	LLLL . LL
19	36	6	0	LLL . LL*L
	36	3	0	RR . L
20	33	2	1	U . RL
21	39	5	0	RR . LLL
22	36	7	0	LLL . RRR*R
23	36	6	0	LL . LLLL
	32	2	0	L . R
24	44	8	1	R*RRLL . RR*R
25	37	3	0	U . RRR
	34	2	0	R . R
26	38	8	0	RR . L*LL*LL*L
27	37	4	0	L . LLL
	34	2	0	U . RR
28	40	5	0	RR . RRR
29	42	5	0	LLLL . L
	46	11	0	RR*RRRR . RRRRR
30	37	4	0	LL . RR
31	33	5	0	LL*L . RR
32	31	6	1	RR . LLLR
	35	6	0	LLL . RRR
33	39	7	1	RR*RL . LLL
34	34	5	1	LLL . RL
35	34	6	0	LL . LLLL
Total	1844	252	15	
Mean	36.9 ± 0.5	5.0 ± 1.0		

*a relic coil at the position indicated

Table XXI

Chromatid length and relational twisting in thirty-seven E chromosomes from root tip cells of T. erectum.

Cell	Chtd. Length	No. of Twists	No. of Changes	Direction of Twisting
1	40	9	1	LLRR . LLLLL
2	34	3	0	RR . R
3	34	4	0	RR . RR
4	49	11	0	RRRRR . RRRRR
5	40	4	0	RRR . R
	42	4	0	RR . RR
6	39	7	0	RRRR . RRR
	36	7	0	RRR . LLLL
7	47	9	1	LRRR . LLLLL
8	50	7	1	LL*LL*L . LR
9	45	5	0	LL . RR*R
10	40	5	0	RR . LLL
11	41	8	1	LRR . LLLLL
12	42	5	1	RL . RR*R
13	44	5	0	LLL . RR
14	41	5	1	RLL . RR
	42	4	0	L . RRR
15	40	4	0	R . RR*R
	40	3	1	R . RL
16	38	3	1	LR . L
17	34	4	0	L . RR*R
	34	3	0	RRR . U
18	45	5	0	LLL . LL
19	45	5	1	RL . RRR
	48	2	0	RR . U
20	41	3	0	R . LL
	41	3	0	L . RR
21	33	4	0	RR . RR
	36	3	1	LR . R
22	38	6	0	RR . LLLL
	42	3	0	R . LL
23	40	2	0	U . LL
	42	3	0	RR . R
24	42	6	1	RRL . RRR
	42	4	0	R . RRR
25	43	3	0	L . LL
	46	4	1	RL . RR
Total	1516	175	12	
Mean	41.0±0.6	4.7±0.3		

*a relic coil at the position indicated

Table XXII

Chromatid lengths*, number of twists, number of changes in direction, and mean intertwist distance in the relational coils of T. grandiflorum and T. erectum microspore and root-tip chromosomes.

	n	Total Length(L)	No.of Twists(T)	No.of Changes	L/n	T/n	L/T+A**
T.grandiflorum							
Microspores							
Group 1	11	1976	330	45	179.6±4.9	30.0±2.7	5.1
Group 2	11	1601	234	30	145.5±1.6	21.3±2.1	5.5
Group 3	11	1359	215	32	123.2±3.1	19.6±1.4	5.0
Group 4	11	926	55	1	84.1±4.3	5.0±1.4	8.4
Total	44	5862	834	108	133.2±5.6	18.9±1.7	6.6
Chr. E	44	1668	266	38	37.9±1.6	6.0±0.6	5.4
Root-tip							
Chr. E	50	1844	252	15	36.9±0.5	5.0±1.0	6.1
T.erectum							
*Microspores							
Group 1	18	3494.4	212	36	194.1±6.8	11.8±1.1	11.5
Group 2	18	2612.8	128	9	145.2±1.3	7.1±0.7	11.9
Group 3	18	2371.2	146	9	131.7±0.8	8.1±0.7	10.0
Group 4	18	2112.0	135	10	117.3±1.8	7.5±0.9	9.4
Total	72	10590.4	626	64	147.0±3.8	8.6±0.5	10.7
Chr. E	72	2954	184	22	40.6±1.1	2.5±0.14	11.5
Root-tip							
Chr. E	37	1516	175	12	41.0±0.6	4.7±0.3	7.1

*Lengths given for T.erectum microspores have been converted from the 2BD lengths given in Table XIX by the use of the 2BD-aceto carmine factor 1.6. All other measurements are from material fixed in alcohol-acetic or in aceto-carmine.

**See footnote to Table XVIII for explanation.

number of somatic chromosomes and, as in microspore relic coils, they were plectonemic in all cases. Positions where the relationally coiled chromatids still retain gyres of the relic coil are marked by asterisks in Tables XX and XXI, which also give the direction of twisting for all root tip E chromosomes of both species. The most obvious difference between the relational coiling in root tips and that in microspore chromosomes is the higher frequency of interstitial changes of direction in the latter (Table XXVII).

The Direction of Coiling

An analysis of direction of coiling at first and second meiotic anaphases, and of relic coils of microspore prophase nuclei, is given in Tables XXIII and XXIV. The mean number of gyres for a complement of five chromatids, the mean number of changes per cell, i.e., 20 chromatids, and the "effective" and true chiasma frequencies are given in Table XXIII. "Effective" here refers to the effect which chiasmata have in permitting or causing changes of direction. If two chiasmata, or a chiasma and an attachment, are very close together (within one half-gyre length) they must act as a unit in this respect. The true chiasma frequency has been obtained from the analyses of camera lucida drawings of chromonemata made by Dr. H. B. Newcombe.

Table XXIII

The Chiasma Frequencies and the Numbers of Gyres and Changes in Direction at Meiotic Anaphase and Microspore Prophase of T.erectum

Material	Stage	No.of Whole Nuclei	Changes in Direction: Total No. of Chromosomes A	B	C	D	E	Mean Gyres per 5 Chtds.	Mean No. Changes per Cell (20 Chtds.)	Chiasma Frequencies: No.of Cells	True Chiasmata	No.of Cells	Effective Chiasmata
Major Coil													
Synaptic													
58-2E-1	A_I	2	6	6	7	4	5	35.6	32.8	-	-	8	14.4±0.7
58-4E-1	A_I	1	6	4	2	3	3	41.2	37.4	2	19.0	10	17.6±0.4
58-5H-3	A_I	1	1	1	1	1	1	35.5	38.0	-	-	12	20.0±0.5
58-5d-3	A_I	1	4	3	2	3	3	45.7	45.8	12	21.3±0.7	12	17.5±0.6
58-5d-3	A_{II}	2	2	2	2	2	2	42.0	40.0				
65-L2-a H+S'35 and	A_I	2	2	2	2	2	2	59.2	46.0	12	14.1±0.8	12	13.3±0.4
Hunter (unpub.)	A_I	2	8	6	5	6	7	57.8	42.6	6	21.8±0.4	20	17.2±0.1
Desynaptic**	A_I	2	2	2	2	2	2	33.1	32.5	-	-	-	10-17
Asynaptic**													
Type 1	A_I	0	1	2	2	2	1	71.0	25.0	-	0	-	0
Type 2	A_I	1	1	1	1	1	1	104.5	42.0	-	0	-	0
Microspore Prophase Relics													
66-T-117	Pro P.G.	5	5	5	5	5	5	38.2	37.6*	-	-	-	-
66-T-72	Pro P.G.	2	2	2	2	2	2	58.5	46.0*	-	-	10	17.2±0.4

* observed figures were multiplied by 4 to facilitate comparison with first and second anaphase.
**Huskins and Wilson'38

Table XXIV

Analysis of Changes in Direction* of the Major and Relic Coils of T. erectum

Material	Mean no. of Intrabrachial Changes per Cell	% Gyres with Intrbrachial Changes	% Possible** Changes Realized	No. Changes Assumed to be Associated with Chiasmata	Remaining No. of Changes	% Gyres with Changes not Associated with Xta. or Attach.
Major Coils	(1)	(2)	(3)	(4)	(5)	(6)
Synaptic						
58-2E-1	24.8	17.4	11.6	14.4	10.4	7.2
58-4E-1	29.4	17.9	11.0	17.6	11.8	7.2
58-5H-3	30.0	21.1	14.1	20.0	10.0	7.0
58-5d-3	37.8	20.6	12.9	17.5	20.3	11.0
58-5d-3(AII)	32.0	19.0	12.1	17.5	14.5	8.6
65-L2-a	38.0	16.0	9.1	13.3	24.7	10.5
H+S '35 and Hunter(unpub.)	34.6	15.0	8.8	17.2	17.4	7.5
Mean		18.1	11.4			8.4
Desynaptic***	24.5	18.5	12.7	10-17	14.5-17.5	11.0-5.8
Asynaptic***						
Type 1	17	6.0	3.4	0	17.0	6.0
Type 2	34	8.1	4.5	0	34.0	8.1
Mean		7.05	3.45			7.05
Microspore Prophase Relics						
66-T-117	29.6	19.4	12.7	17	12.6	8.3
66-T-72	38.0	16.2	9.6	17.2	20.8	8.1
Mean		17.8	11.15			8.2

* Calculations based on the number of intrabrachial changes only, i.e. an average of 8 less (due to the attachments) than the total number of changes observed for the whole complement (Table XXIII

** See text page for method of calculation.

*** Huskins and Wilson '38.

When chiasmata close together in these were counted as one, the frequency was almost identical with that obtained in rough counts made without drawing. The latter method was therefore used in collecting most of the data.

The data from Table XXIII on changes in direction have been analysed (Table XXIV) in a manner somewhat similar to that used by Huskins and Wilson in their Table VII. The following modifications have, however, been introduced: (1) the effective, instead of the true, chiasma frequency has been used in calculating the number of changes assumed to be associated with chiasmata (Table XXIV, column 4), (2) the subterminal attachment of A is omitted as it does not affect the number of changes and (3) whole gyres have been used instead of half gyres.

Knowing the effective chiasma frequency, and assuming that the direction of coiling is random on either side of chiasmata and attachments, as indicated by the data of Huskins and Wilson (1938), the number of changes associated with each can be estimated. Considering only intrabrachial changes, i.e., eliminating changes at the attachment (Table XXIV, column 1), the percentage of gyres with changes (Table XXIV, column 2) varies considerably between different materials. It reaches a mean of 18 per cent in normal synaptic material, but one of only 7 per cent in asynaptic. The means for the same two materials are 8.4 and 7 per cent

respectively when those intrabrachial changes not associated with chiasmata are considered (Table XXIV, columns 5 and 6). From this it seems apparent that intrabrachial changes not associated with chiasmata are approximately proportional to gyre number and that the more extreme variations in frequency of changes are directly related to chiasma frequency.

Changes of direction in the relic coil (Figs. 10, 13, 19 and 20) have been analysed for comparison with changes at first meiotic anaphase (Table XXIV). The mean percentages of gyres with intrabrachial changes are 17.8 per cent for relic and 18.1 per cent for the major coil. We have previously shown that the mean number of gyres at early microspore prophase is only slightly less than at second anaphase (Table VII, 66-T-72). This provides direct evidence that the coils of microspore prophase are really relics of the major coil of meiosis, as Darlington's name for them implies.

The direction of relational twisting has been analysed in order to determine, first, if the direction of coiling is at random across the attachment (Table XXV) and, secondly, whether there is any significant deviation from randomness in the total numbers of right and left twists (Table XXVI). If there were no inherent tendency for a chromosome or a chromosome arm to coil more in one direction than the other in those chromosomes without changes at the attachment,

Table XXV

Direction of relational twists at attachments of microspore chromosomes and root tip E chromosomes in T.grandiflorum and T.erectum

		R.R	R.L or L.R	L.L	R.U or U.R	L.U or U.L	U.U	% of Reversals *
T.grandiflorum microspores	B	16	7	10	2	4	5	21
	C	9	9	7	5	8	6	36
	D	8	11	8	9	1	7	41
	E	4	22	12	1	1	4	58
	Total	37	49	37	17	14	22	
T.erectum microspores	B	5	1	4	35	14	14	10
	C	6	11	7	20	17	12	45
	D	9	12	5	17	14	16	46
	E	9	25	15	8	15	0	51
	Total	29	49	31	80	60	42	
T.grandiflorum root tip	E	7	25	12	4	2	0	57
T.erectum root tip	E	12	19	3	2	1	0	56

* Considering only chromosomes with both arms twisted.

the number of R.R's should be equal to the number of L.L's. If coiling were random across the attachment, we should expect the number of chromosomes with their proximal twists in the same direction (R.R and L.L) to be equal to the number which have a change of direction (R.L and L.R) at the attachment. In both species the B chromosome (with sub-median attachment) has an excess without changes, but the numbers are too small to be reliable in T. erectum. The other chromosomes have more nearly median attachments and all tend towards equality. The attachment therefore appears to be a point of random change in chromosomes C, D and E, but possibly not in B. In chromosomes without changes at the attachment the observed numbers deviated significantly from equality in only one instance, the root tip E chromosome of T. erectum. However, the numbers are too small to be very reliable and when the root tip E chromosomes of both species are considered together, the numbers fit a 1:1 ratio. So far only twists proximal to the attachments have been considered, but when all twists are considered (Table XXVI) there is still a random distribution of right and left twists in all but two of the 14 totals. In T. erectum the B chromosome of the microspores and the E chromosome of the root tips have an excess of right handed twists.

An analysis of intrabrachial changes of direction in relational twisting has been made in microspore and root tip chromosomes of both species. Direction of twisting in the

Table XXVI

Numbers of right and left relational twists in microspore and root tip chromosomes of T. grandiflorum and T. erectum

Microspores

	A		B		C		D		E		Total	
	R	L	R	L	R	L	R	L	R	L	R	L
T.grandiflorum	54	69	74	81	55	61	85	89	121	145	389	445
T.erectum	61	45	68	44	50	58	66	51	86	95	331	293

Root tips

	A-D		E		Total	
	R	L	R	L	R	L
T.grandiflorum	72	55	124	127	196	182
T.erectum	62	52	105	68	167	120

E chromosome has been given in Tables XVIII-XXI, and the frequency of changes, when two or more twists are present in an arm, is given in Table XXVII as the mean number of twists for each change of direction. The latter table shows that the frequency of changes in microspore chromosomes is more than twice that of the root tip E chromosome.

Frequencies of intrabrachial changes of direction have been obtained from both the major and relational coils (Tables XXIV and XXVIII). In order to compare such frequencies it seems advisable to express both in the same terms by transposing the gyres of the major coil into their equivalent number of relational twists. In a plectonemic coil an arm of n gyres will, in the absence of unwinding, give a relational coil of $2n - 1$ relational twists if no changes of direction are present. Each change of direction in the plectonemic coil will reduce by one the equivalent number of relational twists, i.e., a major coil will give a maximum number of relational twists when no changes of direction are present.

The number of possible changes of direction in one arm of a relational coil is one less than the number of twists. Therefore, in an arm with $2n - 1$ relational twists, there will be $2n - 2$ different pairs of adjacent twists, and for a complement of a arms $2n - 2a$, where n is the total number of of gyres in each case. As the number of changes in direction

Table XXVII

Frequency of intrabrachial changes of direction in microspore and root tip chromosomes

	Number of Relational Twists*	Number of Intrabrachial Changes	Mean No. of Twists per Change
Microspores			
T.grandiflorum			
E chromosome	254	38	6.7
All chromosomes	750	104	7.2
T. erectum			
E chromosome	102	21	4.8
All chromosomes	344	56	6.2
Root Tips			
T.grandiflorum			
E chromosome	233	15	15.5
T. erectum			
E chromosome	162	12	13.5

* Considering only arms with two or more twists.

Table XXVIII

Analysis of intrabrachial changes of direction in microspore chromosome arms having two or more relational twists.

No. of Twists per Arm	No. of Arms	Arms with All R's	Arms with All L's	Arms with One Change	Arms with Two Changes	Arms with Three Changes	Total No. of Changes	Mean No. of Twists per Change	Percentage of Possible Changes Realized
				T. grandiflorum					
2	89	34	34	21	0	0	21	8.5	24
3	62	16	17	27	2	0	31	6.0	25
4	31	7	8	13	3	0	19	6.5	20
5	25	3	7	11	4	0	19	6.6	19
6+7	14	3	1	6	4	0	14	6.1	18
8-11	5	2	0	0	3	0	6	7.7	15
				T. erectum					
2	115	44	37	34	0	0	34	6.8	30
3	26	7	8	8	3	0	14	5.6	27
4	9	3	2	2	0	2	8	4.5	30

cannot exceed the number of pairs of adjacent twists, we can express the number of changes as a percentage of the total number of pairs, or, in other words, as the percentage of possible changes realized. In Tables XXIV and XXIX changes in the major and relic coils have been expressed as a percentage of the changes in a relational coil, or coils, whose maximum number of possible changes is 2n - 2a (this is, of course, the number of adjacent pairs of twists). The values obtained (column 3 of Table XXIV, and right hand column of Table XXIX) can be compared directly with the percentage of possible changes actually realized (Table XXVII) in the relational coils studied at microspore prophase.

In Table XXVIII the frequency of intrabrachial changes observed in the relational coils has been analysed by grouping all chromosome arms with the same number of twists. The T. erectum data comprise only arms with 2, 3, or 4 twists. In these an average of 29 per cent of the possible total number of changes is realized. In T. grandiflorum as many as 11 twists were found in one arm. In those with 2, 3 and 4 twists 23 per cent of the possible number of changes is realized. In arms with 8-11 twists only 15 per cent is realized - for the complete range see Table XXVIII. The decrease for each extra twist is small, but the trend is constant and it is steadily approaching the percentage found in the major or relic

Table XXIX

Intrabrachial Changes of Direction in Arms with Two to Eleven Gyres from First Anaphase of T. Erectum (66-T-81-c).

No. of Gyres Per Arm	No. of Arms	Total No. of Gyres	Total No. of Changes	Calculated No. of Poss. Changes	% of Poss. Changes Realized.
2	29	58	2	58	3.5
3	38	114	8	152	5.3
4	64	256	39	384	10.2
5	36	180	29	288	10.4
6	24	204	26	240	10.8
7	50	350	71	600	11.8
8	59	472	84	826	10.2
9	41	369	72	656	11.0
10	9	90	19	162	11.7
11	3	33	6	60	10.0
	353	2126	356	3426	Mean= 10.4

coil of T. erectum (approximately 11 per cent, Tables XXIV and XXIX. It, therefore, seems probable that if sufficient observations could be made at earlier stages in the development of the relational coil before untwisting occurs it might reach the same figure.

A similar analysis of intrabrachial changes in direction in the major coil has been made by grouping together all arms with the same number of gyres, and calculating the percentage of possible changes realized for each group, (Table XXIX). The percentage realized increases from 3.5 to 10.2 as the number of gyres increases from two to four, but there is no appreciable further increase in arms with more than four gyres. It seems apparent, therefore, that in the relational coil the increase in the percentage of possible changes realized in arms with a small number of twists (Table XXVIII) cannot be attributed to a higher frequency of major coil changes in the shorter arms. Chronologically, during prophase, the frequency of changes in the relational coil increases as the number of twists decreases. This must be taken to mean that a larger proportion of twists than changes are being eliminated. The obvious interpretation is that during prophase untwisting or cancellation of relational twists takes place at intrabrachial changes of direction. This occurs when wire models of plectonemic coils are drawn out into relational coils.

DISCUSSION

Combining the previously published observations of Huskins and Smith (1935), and Wilson and Huskins (1938), with those of the present author, the spiralization cycle during gametogenesis in Trillium may be summarized as follows:

The chromonemata at leptotene are thin, beaded, optically single threads which do not appear to have any special or characteristic arrangement. They reach a maximum length at zygotene, during which stage synapsis begins. Contraction is initiated after zygotene and continues through pachytene and diplotene to early diakinesis. Paired chromomeres, which are tightly snyapsed by mid-pachytene, each become bipartite, and by late pachytene the "primary split" has become considerably more distinct than the "secondary split". Further opening out takes place during diplotene as pairs of paired chromatids separate, except where held together by chiasmata.

Between diakinesis and first anaphase elongation of the chromonemata occurs. This is associated with the formation of the major coil, which starts first as a waviness and gradually assumes a more regular form. Sister chromatids are closely appressed during spiralization and form paranemic spirals.

At first anaphase the two chromatids of each dyad fall apart, except at the attachment region. The direction of coiling may change inter- or intrabrachially. At metaphase intrabrachial changes are often, though not always, associated with chiasmata. In some cases each chromatid also shows a "tertiary split", and, as can best be seen at later stages, the half-chromatids are interlocked in a plectonemic spiral.

At metaphase and anaphase a waviness, no doubt homologous to the minor spiral of various authors (p.15), can be seen along the half-chromatids. It does not, however, appear to be a regular spiral in the geometrical sense, but rather an irregular waviness whose adjacent crests are not necessarily of uniform magnitude nor of definite spacial arrangement.

In Trillium there is normally no interkinetic resting stage, and except for a slight contraction of the chromonemata, the major coil of first anaphase is materially unchanged at second division. At second anaphase sister chromatids disjoin, but, as no further splitting has taken place, each is still a half-chromatid plectonemic spiral. During telophase, the major coil gradually becomes obscured by the hydration and katachromatic changes which preceed the formation of the resting nucleus.

When the chromonemata of the major coil reappear at microspore prophase (Fig.29), they are in the form of relic coils, whose gyres are considerably larger than those of the major, but only slightly fewer in number. The major coil has expanded, i.e., the length along the spirals has increased, in the transition to the relic coil. When the chromatids begin to contract, the tertiary split becomes apparent, the relics are seen to be plectonemic and a new small-gyred coil, at first very irregular appears within each chromatid. As the chromatids shorten and thicken, the new spiral grows in diameter, and the gyres of the relic become drawn out to form a relational coil. Further contraction and untwisting of the chromatids gradually eliminate the relational twists, so that by metaphase sister chromatids are, for the most part, free to separate.

Metaphase and anaphase microspore chromosomes have essentially the same structure as those of the second meiotic division (Plate VI). The gyres are, however, more numerous and closer together, and hence more difficult to distinguish. The somatic spiral contains two visibly distinct chromonemata, which, like the half-chromatids of meiosis are irregularly waved. This waviness can be seen in optical cross sections (Fig. 42), as well as in side views, and in neither case does it approach the form of a regular spiral. Observations of the relic coils of the following prophase leave no doubt that the somatic spirals

of metaphase and anaphase are also plectonemic, although this cannot be definitely confirmed by direct observations. Measurements of somatic spirals show that gyre diameter and chromonema length both increase between metaphase and anaphase of the first microspore mitosis.

Preliminary observations of pollen tube mitoses have been made in regard to chromosome structure and mechanics; the results obtained essentially confirm those obtained from studies of microspore mitosis. Here, again, relational coiling is derived from plectonemic relic coils, and the somatic spirals apparently "grow up" by an increase in gyre diameter and a decrease in number (Plate VII).

Length Changes

The value to theoretical cytology of accurate measurements of such obviously important factors in the chromosome spiralization cycle as size changes cannot be overestimated. Meiotic prophase contractions have been measured by several workers (See pages 7-9). Our estimates show that by early diakinesis the chromonema has contracted to one-tenth of its zygotene length. Relatively little attention, however, has been given to chromosome (or chromonema) elongation; although Belar suggested in 1928 (p.171) that an elongation might be related to spiral formation. Wilson and Huskins (1939) demonstrated chromonema elongation during the formation of the major coil. They reported the chromonema to elongate to at least twice its former length (at diakinesis), while our additional measurements indicate that the increase is

probably not less than threefold between diakinesis and first anaphase.

A further elongation occurs in the transition of the major to the relic coil. The gyres increase in diameter and mean length without a corresponding decrease in number. It seems possible that, with the loss of the matrix during the resting stage, the gyres, being no longer restricted by an enclosing pellicle, would tend to expand due to the same resilience that caused them to assume the spiral form in meiosis. Having expanded to a maximum, some physiological change presumably occurs, which starts the reverse process, and the gyres begin to decrease in diameter and mean length, while chromatid diameter increases. The contraction phenomenon and the development of the internal spiral is probably associated with an increased resilience within the chromatids, which tends to straighten them out and unwind them.

Mean chromatid length of early microspore prophases ranges from 450 to 550 μ (Maximum, 652 μ). The length of the contained irregularly-waved chromonema is estimated at about 1000 μ. Mean chromonema length at metaphase is 650 ± 17 μ, and at anaphase is 977 ± 28 μ. However, at both these stages a waviness is present which has not been taken into account in calculating lengths. Consequently, these

values cannot be compared with microspore prophase chromonema length, but would correspond more closely to chromatid length at very early prophase. The high variation in prophase chromatid lengths makes the means underestimates of the true length of earliest prophase. Assuming then that the mean chromatid length of 549±25 μ is an underestimate, we do not feel justified in drawing any conclusions as to length changes between early microspore prophase and metaphase. However, there seems no reason to doubt that there is a very considerable elongation between metaphase and anaphase stages of the microspore division.

In comparisons of chromosome lengths made between individual complements of any one rhizome only 75 per cent are shorter at first anaphase than at diakinesis. The mean anaphase lengths, however, are consistently shorter, which indicated that a slight contraction of the matrix may occur in Trillium. It cannot compare in magnitude with that of Tradescantia (Sax and Humphrey, 1934; Wilson, unpub.). There is a significant decrease, however, in chromosome length between first and second anaphase. Apparently the chromosome contraction, which is indicated between diakinesis and anaphase continues to second anaphase. It should be pointed out, however, that chromosome contraction after first anaphase cannot be interpreted as affecting the formation of the major coil. The apparent chromonema

contraction between first and second anaphase would, presumably, effect the internal structure of the chromatids, i.e., the degree of waviness, and possibly the visibility of the tertiary split.

Maximum meiotic prophase chromonema length has been calculated at 1040 u in T. erectum (Table V). End-to-end chromosome length reaches a minimum at second anaphase (Mean = 72±1.45 μ, Table X). This gives a contraction ratio of 14.4:1, which is in close agreement with the 15:1 ratio found by McClintock in Zea, and Smith (1941) in Diprion. Manton (1939) found a ratio of 18:1 between leptotene and second anaphase in Osmunda. The numerous other reports of contraction ratios (See page 8) are all considerably lower than our 14.4:1 ratio, reaching a low of 4:1 in Marsupials (Koller, 1936).

It has been mentioned above that the mean microspore prophase chromatid length of 549±25 μ is probably an underestimate of earliest prophase length. Mean chromosome length for 15 microspore metaphases is 90.5±3.9 μ. This gives a contraction ratio of 6:1. Using the longest prophase (652 μ) the reduction would be 7.3:1. The 6:1 ratio is, therefore, a conservative estimate; 7:1 would probably not be too high. Sax and Sax (1935) found a 5:1 ratio in Tradescantia, and Patau (1937) 5.5:1 in Collozoum.

Darlington and Upcott (1939) have abstracted some of the published work on chromosome and chromonema size. They find that the measurements of "several workers" agree in giving what they call a "packing factor $\frac{D}{d}$ of 2.0 to 2.5" (D = chromosome diameter, d = diameter of chromonema or of the region occupied by it). As "probably most compatible with observation and with the model" their illustrations of chromosomes representing their value are solid spirals. Our observations on Trillium spirals are not in accord with this conclusion, as both meiotic major and somatic spirals are clearly seen to be hollow. Chromosomes in fresh pollen mother-cells of Trillium mounted in 4% cane sugar solution also show a hollow spiral structure (Wilson, unpub.).

Relational Coiling

Since the chromatids which are relationally coiled at microspore prophase are the product of the tertiary split of meiosis, it is obvious that, in the major and relic coils, the arrangement of the resultant half-chromatids must bear an intimate relationship to the mechanism of relational coiling. Text-figure 1 (a) illustrates diagrammatically how a relational coil can result from drawing out the gyres of a plectonemic coil. Essentially the same process is assumed to transform a plectonemic major or relic coil into relationally twisted chromatids.

During resting stage the chromosomes maintain their anaphase polar arrangement, but by prophase they seem to have developed a stronger mutual repulsion, and to have arranged themselves at the periphery of the nucleus. When arranged thus, the gyres can be seen to be partially flattened out against the nuclear membrane, presumably due to the action of surface tension. Chromatid contraction also tends to straighten out the gyres of the relic coil, at the same time decreasing their diameter and mean gyre length. As the gyres straighten out and the chromatids thicken, relational twists seem to be eliminated by the chromatids slipping around each other. The effect of a viscous karyoplasm on this unwinding process is a moderating one, tending to slow down the attainment of the equilibrium, and leading to hysteresis. As there would be less resistance to untwisting in short arms, they would be expected to lose their twists earlier than longer arms. This frequently happens in the case of B chromosome, which has one short and one long arm.

The loss of relational twists during prophase, by sister chromatids slipping around each other, is probably the most obvious factor in the process of reducing the much interlocked half-chromatids of the plectonemic major coil to parallel rod-shaped chromatids of the microspore metaphase. However, changes in direction of coiling,

decrease in gyre number, and chromatid contractions during prophase, all affect the number of relational twists.

A major coil has the equivalent of $2n-(a+c)$ relational twists, where n is the number of gyres, a the number of arms, and c the number of intrabrachial changes. It is apparent, therefore, that the number of gyres and of changes affects the initial number of relational twists. Gyre number decreases between first and second anaphase, between second anaphase and early prophase, and during prophase contraction. As intrabrachial reversals increase with chiasma frequency, the initial number of relational twists will also be affected by chiasma frequency, assuming that gyre number is constant. Apparently reversals also play a part in the active process of reducing number of relational twists by allowing cancellation or untwisting at the points of change.

For the greater part, chromatid contraction during microspore prophase is not accompanied by any significant change in mean intertwist distance (Table XXII). Since the number of twists is steadily decreasing, we may assume that the twists are being eliminated at the ends, and at reversals, by contraction and untwisting. There is but little evidence that relational twists are lost off the end by a "terminalization" similar to that frequently reported for chiasmata.

Several authors (Darlington, 1936, 1937; Husted, 1936; Sax, 1936; Upcott, 1938) have attributed prophase chromatid relational coiling to the developing internal spiral, which they apparently consider causes an active twisting of the chromatids about each other. We have found no evidence that the amount of relational twisting increases during prophase stages, but have found very conclusive evidence of a continuous decrease. It seems, therefore, that the growth of the internal spiral is associated with the elimination of relational twisting and not with its production, as has been claimed by the above-mentioned authors. Naithani (1937) and Mensinkai (1939) consider that the new internal spiral is instrumental in causing untwisting, but we do not support Naithani's postulate that the chromonemata in the sister chromatids must be coiled in opposite directions (mirror images) in order to untwist the relational coil.

Kuwada (1939) is of the opinion that prophase chromatids each contain two half-chromatids which are in the form of a plectonemic spiral. During prophase contraction untwisting occurs so that by metaphase the two half-chromatids are independently spiralized, or nearly so. This hypothesis seems to make the process unnecessarily complicated, by having untwisting completed one whole division in advance of that at which the chromatids separate.

Then, in order to account for chromatid relational coiling at prophase, he assumes that the half-chromatids which have just finished untwisting by late prophase or metaphase, again become relationally coiled at anaphase.

Abraham's (1939) interpretation is very similar, in that relational coiling is developed during anaphase, but in this case the half-chromatids were separate from their inception and did not have to untwist as in Kuwada's scheme.

Nebel agrees with Kuwada and Abraham that the chromatids in somatic anaphase chromosomes are independently-coiled, but considers that the growth in size of the somatic spiral to the larger relic is responsible for the chromatid relational coiling seen at prophase. In this connection, it is of interest that in Tradescantia the major coil of first meiotic division "grows up" from a smaller-gyred coil, and that in this case the sister chromatid spirals do not twist about each other, but are paranemic (Sax and Humphrey, 1934; Swanson, unpub.). Our observations on Trillium do not lend support to any of these hypotheses concerning the origin or development of the relational coil.

If we assume that the relational coil originates by the drawing out of a plectonemic relic coil, it is apparent

that relational twists would not be in the opposite direction (compensating) to the relic coils, except across changes in direction. In accord with this assumption was the complete absence of compensating relational twists in arms or regions devoid of changes in direction.

These observations do not agree with Darlington's (1935) assumption that relational twists are compensatory. He observes that "relational spirals can sometimes be seen in the same direction as relic spirals", and he therefore has to ascribe non-compensating relational twists to a secondary slipping of the chromatids around one another while the relic coil is still present. This assumption does not appear to be based on observations: "the relic spirals imply (my italics)) the existence of a compensating relational spiral of chromatids in the opposite direction". In any case, this explanation is not valid for Trillium, because the tertiary split, sometimes visible in the major coil, results in a plectonemic spiral. If compensating relational twists are present at a later stage, they must be developed secondarily and not the non-compensating type as Darlington suggests.

Direction of coiling.

The direction of the major coil in synaptic, desynaptic and asynaptic Trillium has been analysed by Huskins and Wilson (1938). They conclude that chiasmata and the attachment

are points of random change, and that in addition a number of changes occurs which is proportional to the number of gyres. Further analysis of first anaphase major coils has given results in complete agreement with Huskins and Wilson. The frequency of reversals in a limited number of second anaphase and microspore relic coils agrees very well with the frequency at first anaphase. Direct evidence is therefore provided that in Trillium the microspore prophase spiral actually is a relic of the major coil of meiosis. In forms having an extended interkinesis it is presumably a relic of the standard coil of the second division.

The direction of coiling of the major spiral of T. kamtschaticum has been analysed by Matsuura (1937a and b). He concluded (1937b) that the number of changes is a function of the length of the chromosome arm (the equivalent of Huskins and Wilson's third factor), and that their origin is apparently fortuitous and bears no relationship to chiasmata. It may be pointed out, however, that this species of Trillium has a very low chiasma frequency. Changes associated with chiasmata would therefore be relatively few, and hence would not significantly alter the expectation based on length. We have analysed the data from Matsuura's (1937 a) Table III for the position of changes in what we call the E chromosome (his A) and have

found the frequency significantly greater between the third and fourth and between the fourth and fifth gyres (from the attachment) than in any other region. There is frequently a chiasma at approximately these positions in both arms of the bivalent.

With the possible exception of the microspore B chromosome, the direction of relational coiling is random across the attachment in both T. erectum and T. grandiflorum. A possible explanation for the apparently anomalous behaviour of the B chromosome has been found by analysing the number of reversals in each of the four chromosomes with non-terminal attachments (Table XXV). The data presented indicate that the greater the discrepancy in length of the two arms, the smaller the percentage of chromosomes with interbrachial changes. If we accept Matsuura's (1935, 1937b) conclusions, that in the major coil the direction is random across the attachment, we should also expect the direction of relational coiling to be random before any untwisting has occurred. However, our data is for later prophase stages, at which time considerable untwisting has occurred. If such untwisting is completed sooner in one or both arms of chromosomes with interbrachial changes than in those without, the relative numbers of the latter would thus be increased. This effect would probably be more apparent in the B chromosome, because of its exceptionally short arm, than in those with more nearly median attachments.

If, as we have postulated, the relational coil is derived from a plectonemic relic, we should expect as many changes of direction in the former as in the latter. However, the proportion of intrabrachial changes in the relational coil is higher than that of the relic (or major) coil, when changes in both are expressed in the same terms, i.e., as the percentage of possible changes realized. Further, the percentage in the relational coil is not constant. In arms with the greatest number of twists it approaches the percentage found in the major coil, but it appears to increase as the number of twists per arm decreases. When a model plectonemic spiral with changes of direction is pulled out into a relational coil, it unwinds from the changes, thus increasing the number of changes relative to the number of twists. This seems to provide the obvious explanation of the difference in frequency of intrabrachial changes between the relational and relic (or major) coils.

Koshy (1933) reports that in Allium all chromosomes change their direction of coiling at the attachment, and that this reversal "is indispensable in the mechanics of mitosis". Presumably, he means that the reversal is indispensable in the elimination of relational twisting. Confirmation of these views is lacking in our observations on Trillium, in which many chromosomes do not have interbrachial changes and yet succeed in untwisting.

We are of the opinion that changes of direction undoubtedly play a part in the elimination of relational twists, but we find no evidence that their role is indispensable to that process.

Husted (1936) has utilized x-ray produced ring and dicentric chromosomes to study relational coiling in microspores of Tradescantia. His conclusions are based on the assumption that such configurations will retain that amount of relational coiling present at the time of reunion following x-ray breakage. However, it is apparent that if untwisting takes place at changes of direction, this argument is valid only for those configurations which were coiled consistently in one direction at the time of reunion. This method, therefore, cannot be relied upon to give any very accurate estimates of the total amount of relational coiling present at the time of reunion, and conclusions based upon this assumption are not, in our opinion, very well founded.

Upcott (1938) has made a study of relic and relational coiling in Hyacinthus microspores and found 95 nucleolar chromosomes "with coils of recognizable direction. With three exceptions these showed no change of direction of coiling within a chromosome arm." Later these three exceptions are ignored, and she states that "the fact that

there is no change of direction in the relic coiling except at the centromere implies that at the previous division, which was meiosis, the internal coiling from which these coils are presumed to be derived is consistent in direction." Changes in direction of the relational coil, she attributes to there being two kinds of relational coiling; the first she assumes compensates for the relic and is in the opposite direction, while "true relational coiling" is brought about by the development of the new internal spiral. This is a restatement of Darlington's (1935) wholly deductive hypothesis.

It would seem to us much simpler to consider relational coiling to be of one type, whose origin may be traced directly to the plectonemic relic coil, and hence back to the major coil of meiosis. Intrabrachial changes being present in the antecedent coils, we should, on this basis, expect changes of direction in the relational coil, and we can, therefore, attach no great significance to the fact that the relational coiling of one region within an arm may be in the opposite direction to relic coils of another region. Neither can we agree with Upcott that the direction of coiling in one division has an indeterminate relationship with that of the next.

Our hypothesis concerning the origin of relational coiling ties up the direction of this coil with that of the relic, and hence, back to the preceding standard or major coil. However, nothing is known about the direction of somatic coiling in successive mitoses. A method of discovering this relationship now seems at hand. Levan (1939) has described colchicine treated Allium root tips in which early prophase chromosomes are sometimes quadripartite instead of bipartite: "The four threads form 2 minor relational spirals which are coiled together into one relational spiral of a higher order". The significance of this observation should be apparent to investigators interested in problems of coiling, for Levan has revealed a method of determining by a single observation the direction of coiling of two successive generations of relational coils. But, since the direction of the relational coils presumably reflects that of their antecedent somatic coil, we can thus indirectly determine the direction of two successive generations of the latter. Unfortunately Levan did not study direction of coiling and his illustrations are not extensive enough to warrant drawing definite conclusions. However, the important thing is that the way is now clear, and this important problem will no doubt be settled in the near future. The same material can also be used to settle the problem of direction of somatic coiling in sister chromatids, about which there is little, if any, definite information.

Theoretical Considerations

The correlation between the diakinesis to anaphase length change and the formation of the major coil has already been discussed by Wilson and Huskins (1939). The observations reported in the present paper bear out the previous conclusion that coiling and elongation occur at the same time. During the period of elongation the chromonema reaches a length of approximately three times its early diakinesis length. Wilson and Huskins have suggested that an increase in chromonema length, occurring within a restricted space, the pellicle, forces the chromonema first into an irregular waviness and then into the regular spiral form. The primary requirements of such a hypothesis of coiling would be (a) the existence of a pellicle, (b) elongation of the enclosed chromonema, and (c) a resilience sufficient to prevent the chromonema from folding instead of coiling. There is now evidence for all these requirements:

(a) It has been generally assumed that the chromonema is, at some stages at least, a duplex structure consisting of one or more chromatic threads embedded in a matrix. If this is so, then a surface boundary or membrane must exist between the matrix and the surrounding medium, and this may be interpreted as a pellicle. The pellicle would be to the chromonema as the nuclear membrane is to the chromosomes and similarly subject to rhythmic changes during the mitotic cycle. Evidence for the matrix itself has been largely observational, but experimental confirmation has recently been obtained.

Sax and Humphrey (1934) consider the presence of a hyaline area about the chromonema to be sufficient evidence of a matrix and pellicle. Kuwada (1935) states that the matrix, in contracting, completes the spiral, thus implying his belief that such exists. In many preparations, particularly aceto-carmine, both matrix and chromonema stain simultaneously, the latter showing as a more deeply stained portion. Experimental evidence concerning the reality of the matrix has been obtained by Hillary (1940) and Painter (1941). Using a modification of Caspersson's (1936) trypsin-lanthanum digestive method, Hillary showed that proteolytic digestion removes the protein but not the thymo-nucleic acid, leaving the chromonemata intact while the halo effect of the matrix disappears. From experiments with alkaline solutions (pH_{13}) on salivary gland chromosomes Painter concludes that the most significant result is "the proof for the presence of a matrix in which the chromonemata are embedded." A chief function of the pellicle may be to regulate the structure of the spiral and insure the evenness of its diameter. In this connection it is of interest that the gyres of a relic coil are often of very unequal size. That the matrix is intimately related to spiralization is indicated by the observation of Dr. Wilson that a mere waviness replaced the usual spiral in material which apparently failed to develop a matrix and pellicle as a result of high temperature (See Figs. 6 and 7; also Wilson & Huskins, 1939).

(b) The evidence presented by Wilson and Huskins, and in the present paper, definitely establish that, in Trillium at least, an elongation of the chromonema is associated with the formation of the major coil.

(c) The strongest evidence for the resilient property of chromosomes is that provided by micro-dissection of Tradescantia chromosomes (Chambers and Sands, 1923) and of spermatogoneal chromosomes of grasshoppers (Chambers (Cowdry, 1924)). Micromanipulation studies of living Trillium chromosomes indicate that they possess a certain rigidity. A reversable elasticity is also indicated by the fact that isolated chromosomes can be pulled out to several times their original length and when released contract to approximately their former size (H. Stern, unpub.).

Our studies on direction of coiling are in accord with previous conclusions that direction of coiling is not inherent in the chromonema nor controlled wholly by an internal force. The data on direction of major and relational coiling are incompatible with hypotheses such as those of Darlington (1935) and MacKnight (1940), which assume coiling to be produced by an internal torsion with its direction controlled from the centromere. Other theories of spiralization based on rotation of chromosome ends (Koshy, 1933; Abraham, 1939; Coleman and Hillary, 1941) or of the centromere (Catcheside, 1932) are similarly discredited, not only by their failure

to explain changes of direction, but as Darlington (1939, p.56) has pointed out, by the fact that the rate of coiling is the same in long arms as in short. (If coiling were by rotation we should expect short arms to coil more quickly than long arms.) It also seems highly probable that coiling by rotation would give plectonemic sister chromatid major coils and not paranemic, as is usually observed in Tradescantia and Trillium.

The probable role of changes of direction in the elimination of relational twisting has already been discussed, and it seems logical that the more changes in an arm of a given number of gyres the easier will be the untwisting. Therefore, since chiasma frequency affects the number of intrabrachial changes of direction, it will also affect the mechanics of chromatid untwisting at microspore prophase. It is therefore suggested, speculatively, that a high cross-over value might speed up the microspore mitosis sufficiently to give a physiological advantage at germination to the microspores with a high recombination. If such a selection actually does occur, it would tend to maintain a higher degree of heterozygosity than would otherwise exist in a plant such as Trillium which, presumably, is largely self-fertilized.

It has been stated above that the somatic spiral is considered to grow up by an increase in gyre size and decrease in gyre number. When this is tested with a model

it is apparent that gyres proximal to the attachment get their extra length at the expense of the distal ones. However, since gyre size seems to remain more or less uniform throughout, this must mean that each gyre expands in proportion to the number lost at the end. Hence, each time a gyre is lost the redistribution will extend all down the line to the last gyre at the other end. However, it appears in the model that redistribution can occur from changes as well as from the end. It seems probable, therefore, that changes of direction may play a part in the formation of the spiral as well as in its untwisting.

While it seems highly improbable that a regular circumnutation of the chromatids accompanies coiling, we cannot, at present, eliminate the possibility that they rotate on their own axis. Our observations indicate that pairs of half-chromatids at meiosis form plectonemic spirals. However, since we do not know at what stage the plane of the tertiary split is determined nor its direction, we can only speculate as to how and when the plectonemic condition arises:

The plane may be determined before the initiation of major coiling, in which case the cleavage could be either spiral or straight. If it were spiral, untwisting might occur, and if it were straight twisting could occur. Thus, in either case, the half-chromatids could be either (a) untwisted, or (b) twisted when coiling is initiated.

If the former, they must become twisted during coiling to produce a plectonemic coil. If the latter, further twisting may or may not be required, or some may be eliminated, depending upon the original amount present. If twisting is present when coiling begins and is incorporated into a plectonemic spiral, the direction of the two must agree. (This is just the reverse of the direction of the relic determining that of the succeeding relational coil.)

If the plane of the tertiary split is determined after coiling, it must, in order to produce a plectonemic spiral, be perpendicular to the long axis of the spiral and rotate through 360° for each complete gyre of the major coil (Text fig.1).

Owing to the fact that with the optical equipment now in general use half-chromatids are near the limits of visibility, it is very difficult to obtain indisputable evidence of their structure and relationships. The final solution of the problem will, therefore, probably have to await the further development and application of new equipment or techniques. The pioneer spectrophotometrical methods of Caspersson (1936), the further use of polarized light (Schmidt 1936 a and b, 1937) and of the newly developed electron microscope are all promising new fields of investigation in chromosome metabolism, behaviour and submicroscopic structure.

It is the opinion of several investigators (Husted, 1938; Koller, 1938; Darlington, 1939, p.55) that the somatic spiral "grows up" from a many-gyred spiral of small diameter at early prophase to one with fewer but larger gyres at metaphase. Our data indicate a decrease in gyre number from something over 600 at early microspore prophase to about 120-130 at metaphase or anaphase. There is a corresponding increase in gyre diameter from considerably less than one micron to almost three (2.77).

Several authors (Kaufmann, 1926a; Koshy, 1937; Naithani, 1937; Kuwada, 1939) have suggested that the chromatids (of mitosis) are twisted at the time of their inception. In accord with this suggestion we consider it highly probable that somatic spirals are plectonemic, as their relics clearly indicate. Then, regardless of the time when the split becomes apparent, if the resultant half-chromatids form a plectonemic spiral, any reduction in number of gyres is the equivalent of a loss of relational twists in the derived relational coil. On this interpretation it is clear that the unwinding process, begun with the development of the somatic spiral at one prophase, would continue until the late prophase or early metaphase of the following mitosis. Therefore, it seems not unreasonable to suggest that the whole spiralization cycle can be interpreted as a continuous unwinding process necessitated by the fact that the plane of the split which produced the chromatids was a spiral cleavage.

SUMMARY AND CONCLUSIONS.

The chromosome spiralization cycle in Trillium has been studied in meiotic, microspore, pollen tube and root-tip divisions. The various types of spirals (major, minor, somatic, relic and relational) have been observed and their structure, transformations and inter-relationships investigated. These, together with measurements of chromosome, chromatid and chromonema length changes have led to the following conclusions:

1. Elongation of the chromonema occurs between early diakinesis and first anaphase, and between second anaphase and microspore prophase, and between metaphase and anaphase of the microspore mitosis. Contraction occurs between zygotene and early diakinesis, between first and second anaphase and during microspore prophase.

2. Early diakinesis to anaphase chromonema elongation is associated with the formation of the major coil, and its transition into the relic coil of the microspore prophase is accompanied by a further elongation.

3. Microspore chromatids at metaphase are about one seventh their early prophase length. This is the highest contraction ratio yet reported for somatic chromosomes.

4. Mean chromonema length and gyre number in microspore anaphase chromosomes are more than twice that of meiotic anaphase chromosomes of approximately the same mean length. The gyres of the somatic spiral are thus more tightly "packed".

5. Direction of coiling of major, relic and relational coils has been analysed. Frequency of reversals at first and second anaphase and in microspore prophase relics agree closely. This indicates that the prophase spirals are really relics of the major coil as their name implies.

6. Changes of direction fall into three categories; (1) those associated with attachments, (2) those associated with chiasmata, and (3) those probably of fortuitous origin, not associated with attachments or chiasmata. The frequency of the third category is proportional to the number of gyres.

7. The frequency of interstitial changes of direction is higher in microspore prophase than in root-tips, presumably because factor (2) is inoperative in the latter.

8. There are very few data that could possibly be taken to indicate that chromosomes may have an inherent directional pattern of coiling.

9. An irregular waviness or corrugation, frequently referred to as a "minor spiral" is present in the chromonemata of the large-gyred spirals of both meiotic and microspore chromosomes.

10. The somatic spiral in microspore chromosomes develops from a small sized, many-gyred spiral at early prophase to one with fewer but larger gyres at metaphase and anaphase.

11. Microspore anaphase chromosomes are considered to have essentially the same spiral structure as second meiotic chromosomes, i.e., a single coil (but not necessarily single-stranded) rather than two or more independently coiled chromatids, as is frequently maintained.

12. The tertiary split, sometimes visible as early as first metaphase, results in the half-chromatids being associated in the form of a plectonemic spiral, which persists as such to microspore prophase. Somatic prophase relics are also plectonemic.

13. At prophase the gyres of the plectonemic relic coil become partially straightened out to form a relational coil, whose twists are in the same direction as their antecedent relic coils.

14. During microspore prophase the number of relational twists decreases with chromatid length. The development of the new somatic spiral seems to be associated with the <u>elimination</u> of relational twisting and not with its <u>production</u>, as is frequently maintained.

15. Relational twists are apparently eliminated at changes of direction, and by contraction and untwisting of the chromatids.

16. There is evidence indicating that relational twists are lost at an earlier stage (based on chromatid length) in T. erectum than in T. grandiflorum.

17. It is suggested that the whole mitotic spiralization cycle can be interpreted as a continuous unwinding process whose purpose is to reduce the interlocked halves of a plectonemic spiral into parallel, freely-separable chromatids. This is accomplished by reduction in gyre number, chromatid contraction, and the loss of relational twists at prophase by cancellation across changes of direction and by chromatids slipping around one another.

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DESCRIPTION OF PLATES

All photomicrographs except fig. 29 are taken from 2BD-crystal violet (Plate I) or aceto-carmine preparations (Plates II-VIII). Fig. 29 is from a root tip smear stained by the Feulgen method. All magnifications are 1000 X unless otherwise stated.

PLATE I

Figs. 8 and 9 are from T. grandiflorum, the remainder from T. erectum.

Fig. 1. A diakinesis cell from 58-5d-3 showing an early stage in coiling.

Fig. 2. A late diakinesis or early metaphase cell from 58-5d-3 showing coiling about half completed. Note unpaired A chromosomes.

Fig. 3. An early first anaphase cell from 58-5d-3 showing coiling completed.

Fig. 4. Second anaphase from 58-5d-3.

Figs.5 and 8 First anaphase from T. grandiflorum. Note tertiary split.

Figs.6 and 7 Abnormal first metaphase and first anaphase without major coiling. See text, page 111. Note the tertiary split near lower left corner of fig. 6. (Magnification 1450 X)

Fig. 9. A first anaphase from desynaptic T. erectum material (Huskins and Wilson, 1938) showing interlocked half chromatids spirals formed by the tertiary split. (Magnification ca. 1200 X)

PLATE II

Progressive stages from early prophase to metaphase in microspores of T. erectum. Lengths given were calculated from camera lucida drawings of aceto-carmine preparations. Figures 10-13 are from 66-T-117 and 14-18 are from 66-T-72.

Figs. 10, 11 and 12. Early prophases (565μ, 498μ and 463μ respectively) showing relic coils. In fig.11 only the upper level of the nucleus is in focus.

Fig. 13. Prophase (383μ) showing relic coils and tertiary split (plectonemic spiral). The gyres are somewhat more straightened out and the twisting of the chromatids about each other is now apparent.

Figs. 14-16 Progressively later stages (259μ, 193μ and 143μ respectively) with fewer relics and relational twists. The split is now very clear. Figs. 15 a and b are different levels of the same cell.

Fig. 17 Late prophase (123μ). Note especially the smaller chromatid diameter ("differential reactivity") at one end of the C chromosome. Cf. Darlington and La Cour (1938).

Fig. 18. Metaphase (79μ). Relic coils and relational twists have been completely eliminated. Note indications of spiral structure within the chromatids.

PLATE III

Progressive stages from prophase to metaphase in microspores of *T. grandiflorum*

Fig. 19. Prophase (346μ) showing chromosomes in the polar arrangement of the preceding anaphase, relic coils and tertiary split. (69-G-104)

Fig. 20. Later prophase, (ca. 290μ) relics partially straightened out showing them to be plectonemic. (69-G-118)

Figs. 21-23. Progressive stages of contraction (187, 131 and 103μ respectively) in cells from groups 1, 3 and 4 respectively of Table XVIII. Note the high variation in intertwist distance. (69-G-104)

Fig. 24. Metaphase (79μ). Note failure of attachment region to stain and indications of spiral structure, especially in the A and C chromosomes. (69-G-100)

PLATE IV

Figs. 26 and 29 are from T. erectum, and the rest are from T. grandiflorum.

Fig. 25. Metaphase (63μ) from 69-G-104. This microspore metaphase is the shortest one measured.

Fig. 26. Late metaphase root-tip chromosomes, showing relational twisting (Length of a haploid complement 144μ). (Magnification 1450 X)

Figs. 27 and 28. Microspore prophases showing relic coils, tertiary split and wavy or corrugated chromonema. Note the changes of direction in the relic coil in Fig. 27. (Magnification of Fig. 27, 920 X)

Fig. 29. Early microspore prophase, showing chromonemata and relic coils.

Fig. 30. Binucleate microspore. Chromonemata and relic spirals can be seen in the generative nucleus (Left).

PLATE V

Microspore of T. grandiflorum (69-G-100) at early, mid and late prophase stages, showing somatic spirals and relational coiling.

Fig. 31. Early prophase.

Fig. 32-35. Mid prophase (Chromatid lengths around 200μ).

Fig. 36. Late prophase (Chromatid length 142μ).

PLATE VI

Somatic spiral structure in microspore chrosomes of T. grandiflorum.

Figs. 37-39. Microspore metaphases showing spiral structure.

Figs. 40-42. Microspore anaphases showing spiral structure. The optical cross sections in Fig. 42 show the somatic spiral to be hollow.

PLATE VII

Prophase to metaphase stages of the generative cell in pollen tubes of T. erectum cultured in sugar solution.

Figs. 43-46. Prophase stages showing relic coils. (See also lower right-hand corner of Fig. 50)

Fig. 47. Late prophase after the relic coils have been lost. Note the cross-striations, indicative of the internal spiral.

Figs. 48-50. Metaphase configurations; Fig. 48, relational coiling; Figs. 48 and 49, somatic spirals.

PLATE VIII

Giant microspore with nine chromosomes from T. grandiflorum. The chromatids are well separated and the relational coiling exceptionally clear.

PLATE I

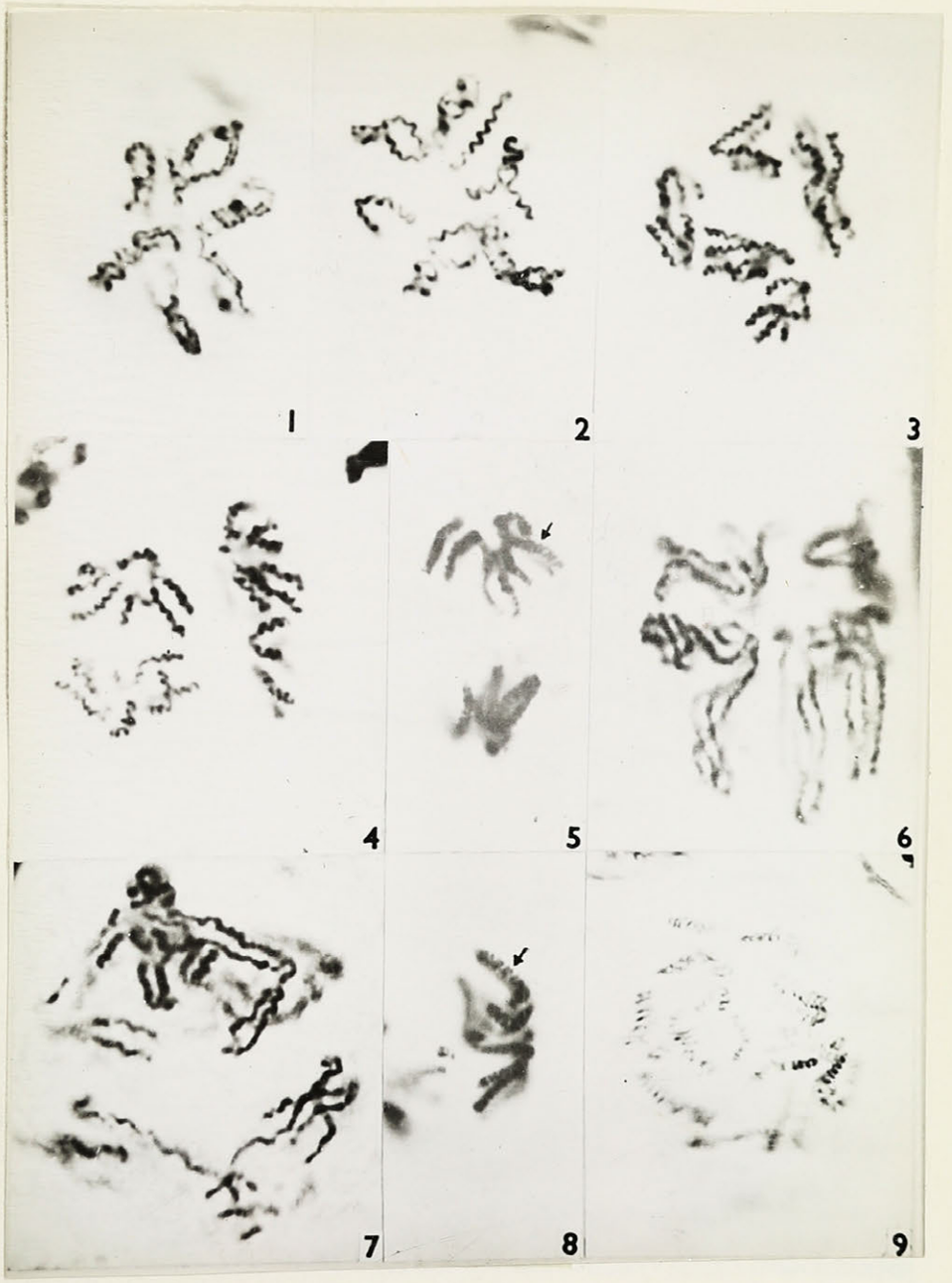

PLATE II

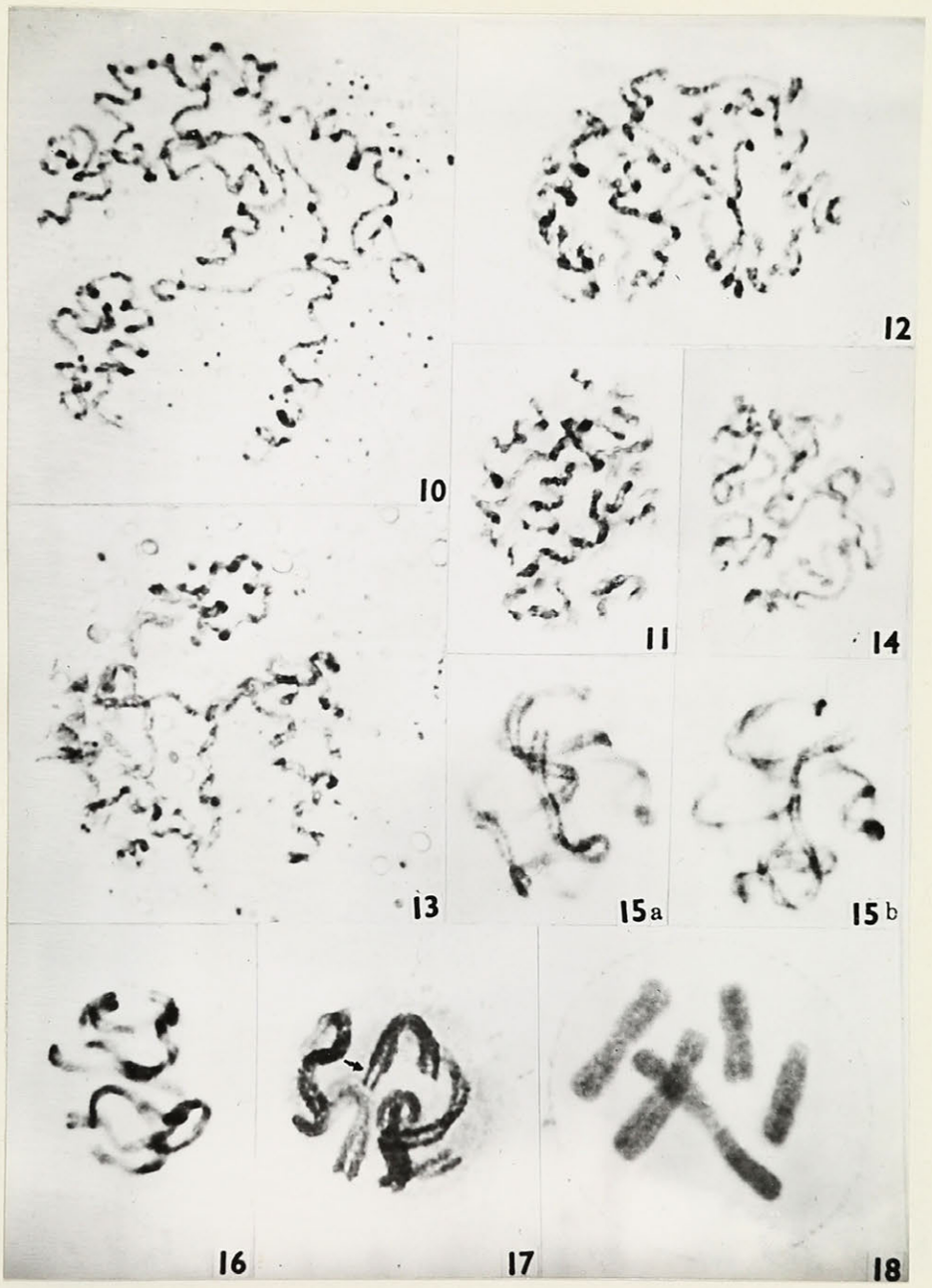

PLATE III

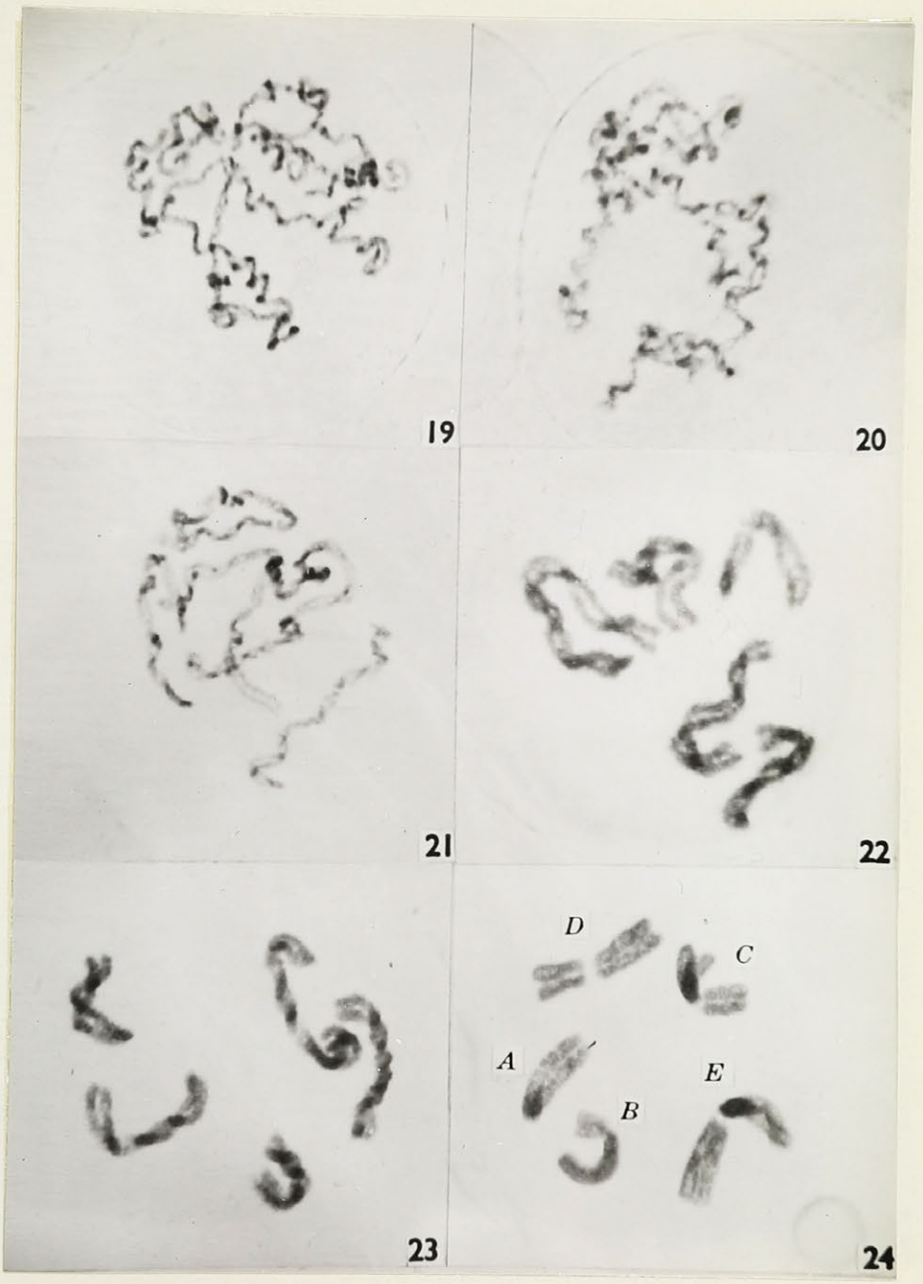

PLATE IV

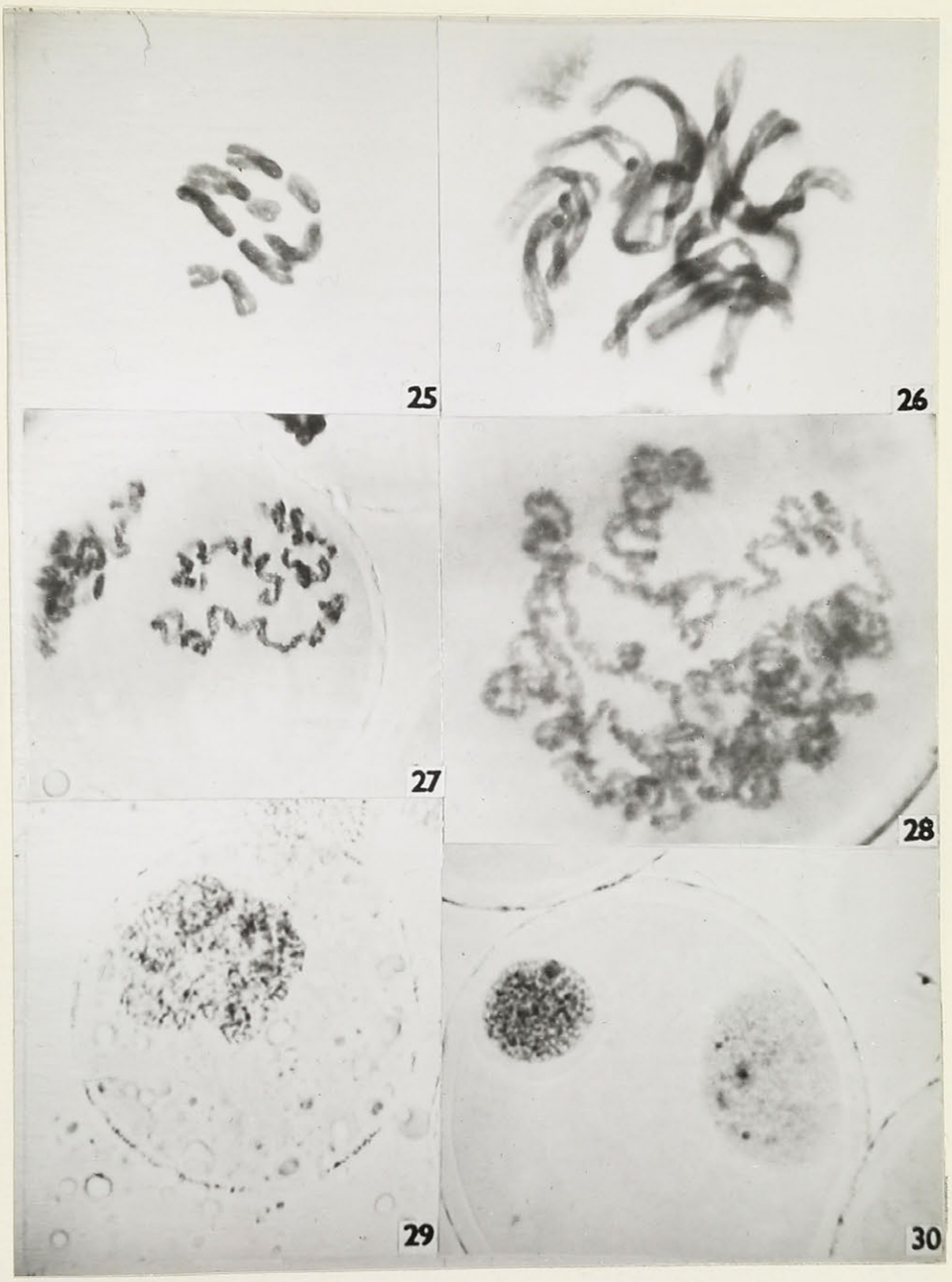

PLATE V

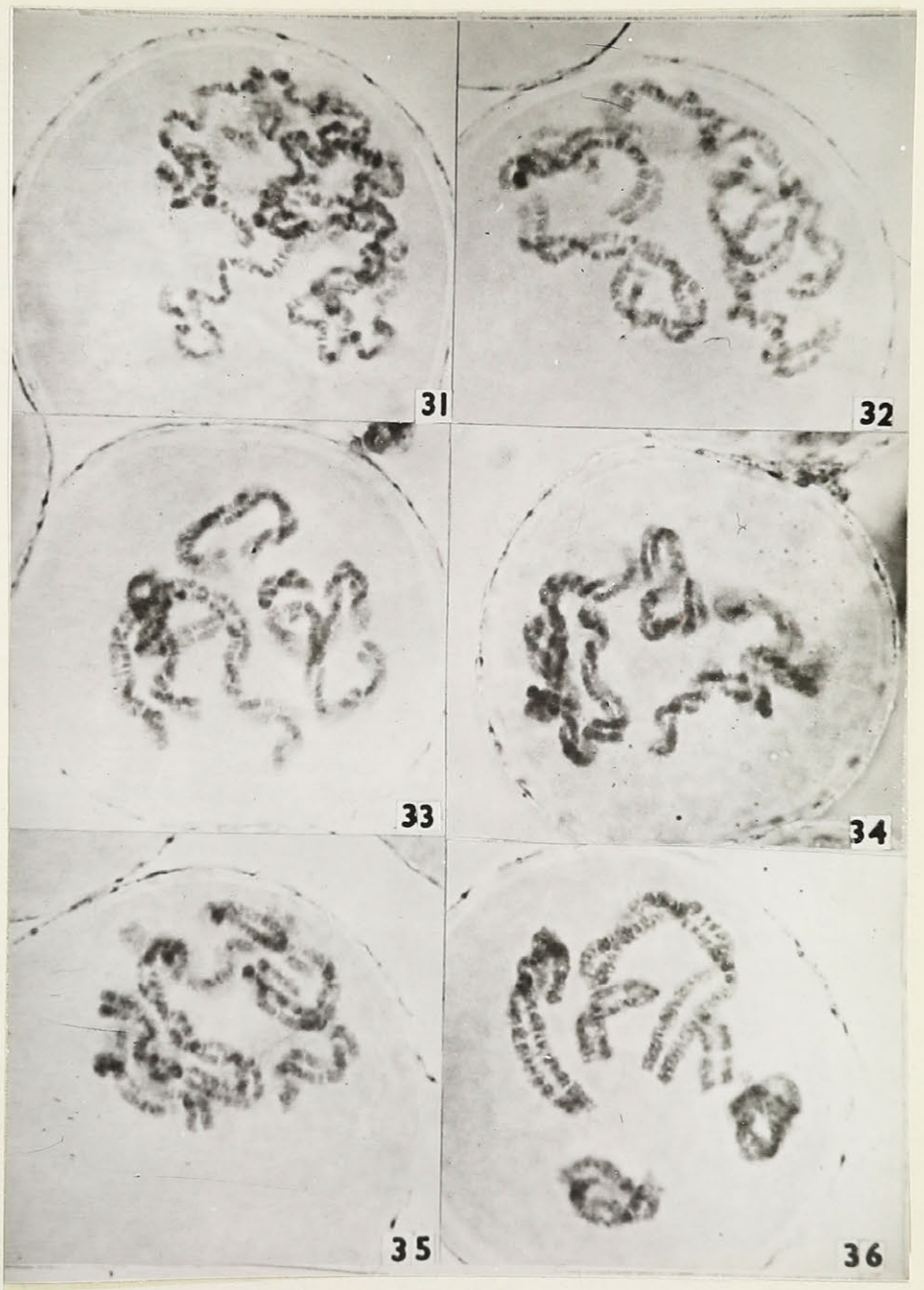

PLATE VI

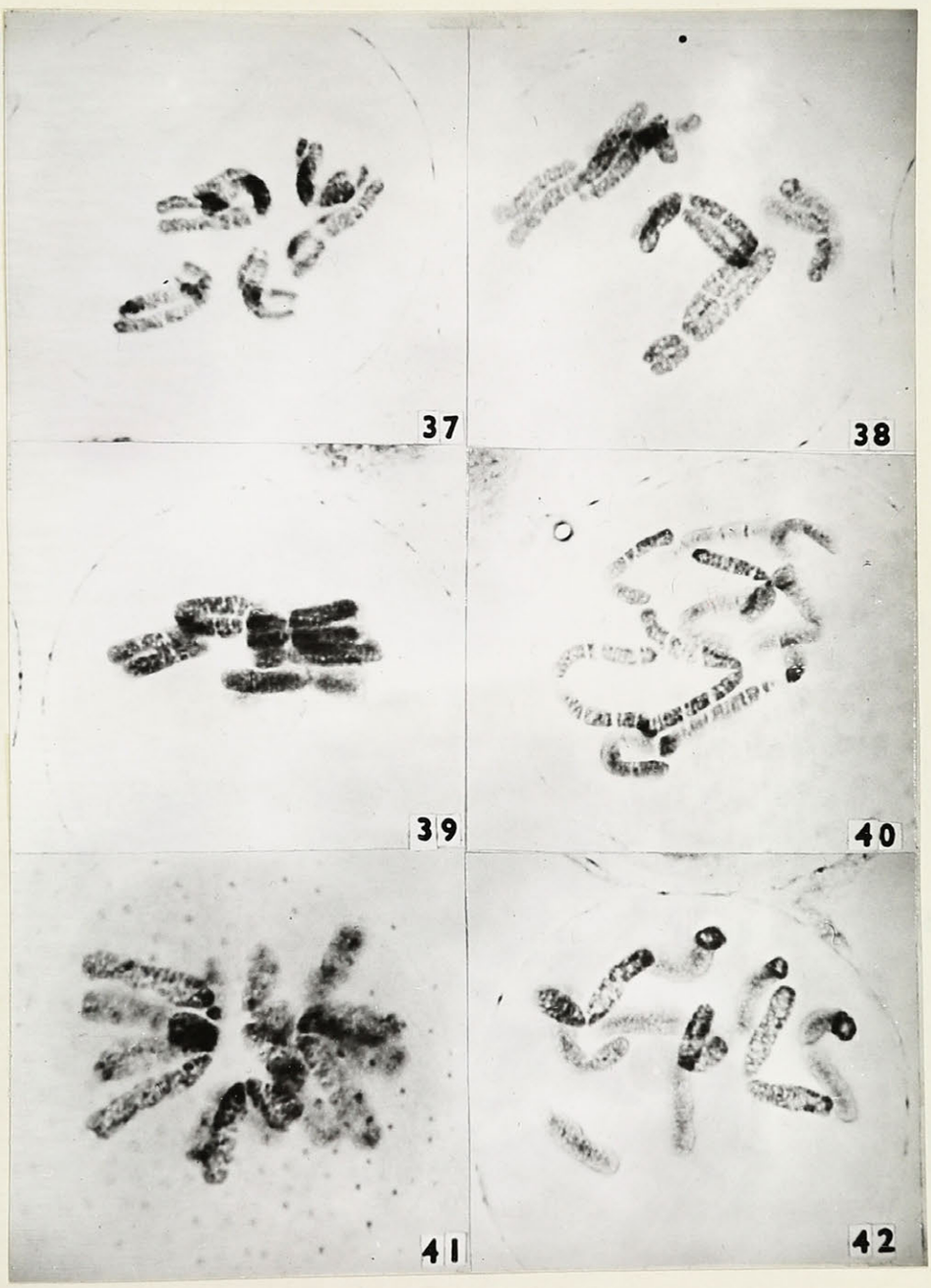

PLATE VII

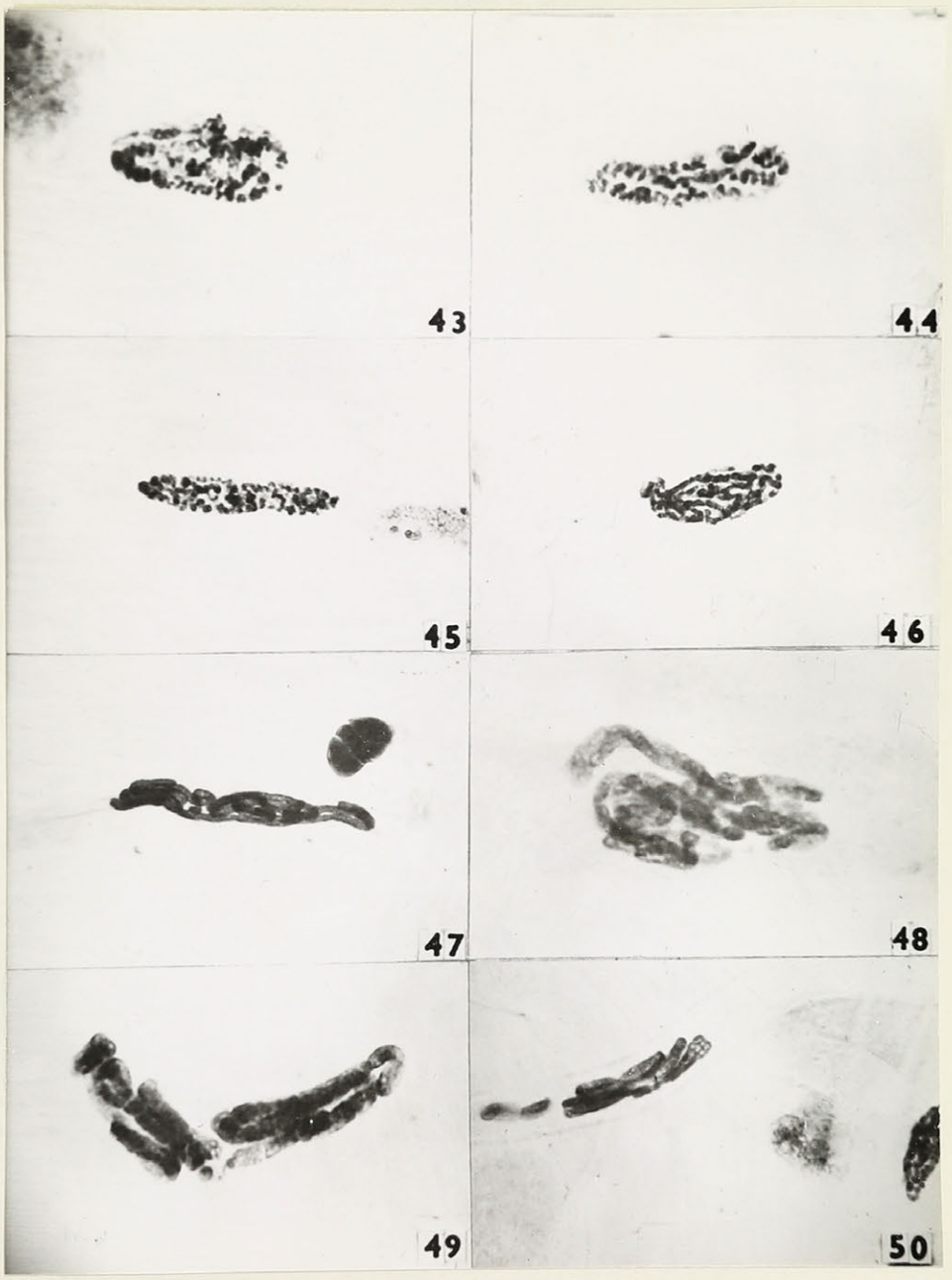

PLATE VIII