The Ontogeny of Chromosome and Chromonema Spirals
A Re-evaluation

G. B. Wilson and P. G. Coleman

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Introduction

The question of the ontogeny of the several observable chromosome and chromonema spirals has led, in the past, to a considerable volume of literature. No attempt will be made here to review this in detail. Most of the opinions and critical data have been presented by Kaufmann (1936), Darlington (1937), Kuwada (1939), Nebel (1939), and Sparrow, Huskins and Wilson (1941). The main purpose of this paper is to re-evaluate the compression hypothesis as originally elaborated by Wilson and Huskins (1939) following Sax and Humphrey's (1935) suggestion that coiling may be the result of compression of the chromonema within the matrix. This hypothesis may be contrasted with the more generally accepted one that the visible coils are a direct result of torsion at the molecular level (see especially Darlington 1935, 1937).

Materials and methods

Some new observations and mensuration data on somatic chromosomes from Allium root tips and Tradescantia microspores will be presented. The preparations used were not originally made for our current purpose. The Allium slides were made from root tips treated with either Penicillin or Acti-dione and were, for the most part, prepared by Dr. M.E. Hawthorne. The Tradescantia slides were prepared by Mrs. G.B. Wilson during the winter of 1939-40 from X-rayed material. Some observations have also been made on chromosome relational coiling at pachytene of Rye and Podophyllum.

Our methods of analysis were primarily photographic. Considerable preliminary investigation led to our final method which was essentially as follows: Thirty-five mm photomicrographs were taken on microfile which was developed in a continuous tone fine-grain developer. Prints were made on Kodalith which was also developed in a continuous tone developer. The prints were held against a lighted background and measurements made directly from them. Details were checked by re-examination under the microscope wherever deemed necessary. The formula used for calculating the length of a coiled chromonema was that given by Sparrow, Huskins and Wilson (1941). The largest single

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1 Respectively Associate Professor of Botany and Experiment Station Photographer, Michigan State College, East Lansing.

Contribution 52-13 from the Department of Botany and Plant Pathology, Michigan State College.
source of error arises from the difficulty of making accurate measurements of gyre diameter especially at prophase. However, all such measurements were carefully checked and are the averages of a number of individual readings.

**Observations and discussion**

1. The helical coils

   a. In meiosis

      i. The major coil: It has long since been established beyond any reasonable doubt that the chromonemata are coiled in the form of a helical spiral from first metaphase to interkinesis. If interkinesis is missing, as it is in some cases, this same coil persists until the inception of the first mitotic division after meiosis when it becomes unraveled as a relic coil. Following the usage of Huskins and Smith (1935) we shall call this spiral the major coil. Although studies on the inception of this coil have been confined mostly to the large chromosome plants, it seems probable that there are in general two systems of development which may be referred to as the Trillium and Tradescantia types since these two organisms have provided the most detailed information. In the Trillium type the major coil begins as a waviness at early diakinesis and gradually becomes a more or less regular helix by first metaphase. During this period both chromosome length and diameter remain approximately constant while the chromonema increases in length. In the Tradescantia type the coil first becomes obvious as a small gyred spiral at early diplotene which develops by losing turns and increasing in diameter. During this period chromosome length obviously decreases but what if any change may occur in chromonema length has yet to be determined (see Swanson 1942). Which one of these systems may be the most common or whether there are others distinct from these two are currently unanswerable questions and will remain so until more detailed studies of coiling have been made in a wider range of organisms.

      In both cases there is considerable evidence that the direction of coiling is inconsistent both within and between arms. Detailed analyses of Trillium itself have indicated that the direction of coiling is random across any point of mechanical interference (Huskins and Wilson 1938; Matsuura 1937, and Wilson and Hutcheson 1941).

      ii. The minor coil: The majority of Tradescantia workers, following Fujii 1926, have described a secondary coil at first metaphase and anaphase, the gyres of which run at right angles to those of the major spiral to give a double coiled structure. This secondary coil is generally known as the minor spiral. There appears to be a rather conspicuous lack of unanimity concerning both its origin and fate. Some
(e.g. Darlington 1937) appear to consider it to be primarily developed and to be causally related to the subsequently formed major coil. Others (e.g. Kuwada 1936) would seem to consider it to be secondarily derived and, by implication at least, more or less independently determined. As to its fate, the most generally held opinion seems to be that it becomes the standard of the second division (where there is an interkineses between the two divisions) and is homologous with the standard coil of mitosis (see Nebel 1939). The McGill group (see especially Huskins 1937) have quite consistently expressed doubt concerning the reality of the minor coil as a true helix but admit a zig-zag appearance in Trillium. This distinction may be labelled as mere hair-splitting but there are or may be significant physical and geometrical differences between an essentially one-plane waviness and a three dimensional helix.

b. In mitosis

i. The standard coil: That somatic chromosomes at metaphase and anaphase also contain their chromonemata in the form of helical spirals can no longer be doubted. In very few cases, however, can direct studies of these spirals be made so that most of our information has been obtained more or less indirectly through analysis of relic and relational coiling (Sparrow, Huskins and Wilson 1941; Sparrow 1942). Development of this spiral appears to be similar to that of the Tradescantia type of major coil though inception is relatively earlier.

During the past two years occasional preparations of Allium root tips have revealed spiral structure at metaphase and anaphase with sufficient clarity to allow partial analysis of the properties of the coil especially with respect to direction of coiling (Pl. IV, figs. 1, 2 & 3). These preparations have been examined with two questions in mind:

1. Is the coiling direction consistent within an arm?
2. Do sister chromatids coil independently or not?

Since it has proved impossible to trace the coils accurately for more than a few gyres, no quantitative answers can be given but, qualitatively speaking, there is no doubt that intrabrachial reversal do occur and we would estimate a frequency of not less than one per arm and there is also no doubt that sister chromatids are at least partially independent in coiling direction.

Some preparations of the first microspore division of Tradescantia reflexa were also found to reveal fairly clear spiral structure at stages ranging from early prophase to anaphase (Pl. IV, figs. 5 & 6). Although accurate gyre counts could be made for substantial chromosome segments, the direction of coiling was difficult to determine with satisfactory certainty owing to the small gyre size in prophase and the masking effect of the matrix at later stages. None the less it is again clear that intrabrachial reversals occur with appreciable frequency.
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Table 1. A comparison of prophase, metaphase, and anaphase chromosome and chromonema lengths, and chromosome volumes during the first microspore division of Tradescantia

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Pro.</td>
<td>18</td>
<td>203-10.1</td>
<td>166-5.7</td>
<td>1.17-.04</td>
<td>645</td>
<td>.315</td>
<td>214</td>
</tr>
<tr>
<td>Meta.</td>
<td>18</td>
<td>115-2.8</td>
<td>95-2.3</td>
<td>1.96-.03</td>
<td>594</td>
<td>.194</td>
<td>347</td>
</tr>
<tr>
<td>% change from Pro.</td>
<td></td>
<td>-43%</td>
<td>-43%</td>
<td>+67%</td>
<td>-8%</td>
<td>-38%</td>
<td>-61%</td>
</tr>
<tr>
<td>Ana.</td>
<td>7</td>
<td>95-3.5</td>
<td>84.5-5.0</td>
<td>1.97-.03</td>
<td>534</td>
<td>.178</td>
<td>289</td>
</tr>
<tr>
<td>% change from Meta.</td>
<td></td>
<td>-17%</td>
<td>-11%</td>
<td>-10%</td>
<td>-8%</td>
<td>-17%</td>
<td></td>
</tr>
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* Calculated from means of chromosome lengths, diameters, and gyre numbers.

The fact that fairly accurate gyre counts as well as diameter and length measurements could be made has enabled us to calculate changes in chromosome lengths and volumes and chromonema lengths from late prophase to anaphase. A summary of these data is given in Table 1 in which the grouped prophase measurements and calculations are compared with those from metaphase and anaphase. Regardless of the exact progression of events there can be little doubt that the following changes occur:

1. Chromosome length decreases.
2. Chromatid diameter increases, at least to metaphase.
3. Chromatid volume increases to metaphase and then decreases.
4. Chromonema length decreases very slightly.

With regard to chromonema length change, we are of the opinion based on fragmentary measurements, that there is little consistent length change during prophase at least after initial development of the spiral. Calculations of chromonema length changes between metaphase and anaphase are not as extensive or as complete as we could wish. However, there does not appear to be any elongation such as that described by Sparrow (1942) for Trillium microspores.

ii. Relic coiling: That the rather loose helical coil found at the beginning of a mitotic prophase is a relic of the helix of the previous division is now generally accepted. It therefore follows that the direction of coiling in the relic spiral is a reflexion of that of the helix from which it is derived. Sparrow, Huskins, and Wilson (1941) analyzed the relic coil of the first microspore division of Trillium and found a high correlation between the frequency of intrabrachial reversals in it and the major coil from which it originates. Analysis of relic coils in somatic figures from root tips also revealed intrabrachial reversals. It is therefore our considered opinion that the direction of coiling in the “mitotic” helix is subject to fortuitous reversals within arms.
2. Relational coiling

a. Chromatid relational coiling

It is a matter of common observation that the two chromatids of a somatic chromosome are wound about each other in a relational coil. Sparrow, Huskins, and Wilson (1941) showed that:
1. The number of relational twists diminish during prophase.
2. There is one twist for each turn of the relic coil.
3. The direction of twisting corresponds to that of the relic coil.
4. The relational twisting originates at the same time as the coil from which the relic derives and probably by the same mechanism.

The relational coil also is not necessarily consistent in direction either between or within arms.

b. Chromosome relational coiling

Darlington (1937) assumes as part of his general theory of coiling and chiasma formation that homologous chromosomes become relationally twisted presumably shortly after or coincidently with pairing. This assumption has been rather widely and perhaps uncritically accepted as a fact despite meager observational evidence. That such twisting does occur, however, we do not doubt especially since we have recently encountered very clear cases in mid-pachytene of Podophyllum (Pl. IV, fig. 4). The twisting becomes obvious well after chiasmata are apparent and many arms with one or more chiasmata are devoid of twists. Careful examination of equivalent stages in rye reveals little or no true twisting. It therefore appears to us that chromosome relational coiling is variable in degree and fortuitous in occurrence. The peak development of chromosome relational coiling seems to be subsequent to both pairing and chiasma formation.

3. The hypotheses

Many suggestions as to the origin of the various coils have appeared in the literature but only two are readily subject to experimental and statistical testing. Our discussion will therefore be confined to these two.

a. The torsion hypothesis

Perhaps the most widely accepted schemes are based on the induction of internal torsion through spiral orientation of the molecular structure. The simplest and most straightforward version is that proposed by Darlington (1935, 1937) who assumes a molecular spiral consistent for at least a chromosome arm which in turn determines the visible helices in some cases and relational coiling in others. The expected properties of spirals formed by this mechanism may be to some extent a matter of argument. However, intrabrachial reversals in either the helical or relational coiling would be difficult to explain as would the positive correlation of chromonema elongation with coiling.
Also the whole question of alternate determination of helical and relational coiling becomes highly confusing.

b. The compression hypothesis

Wilson and Huskins (1939) following the lead of Sax and Humphrey (1935) elaborated on a general hypothesis of coiling which had as its basis the idea that coiling may be a direct consequence of a differential length change between the matrix and its enclosed chromonema. The expected properties of such a compression spiral allow for both reversals in direction and chromonema elongation during coiling. Also chromatid relational coiling poses no serious problem. A single thread under compression tends to rotate on its own axis to give one twist for each turn of the helical coil. If the cleavage plane is already determined prior to coiling then the half threads lie parallel when coiled and become relationally twisted when the helix is pulled out. If two threads are separate when coiled they will tend to rotate about each other but this is likely to prove mechanically difficult since the free ends must describe orbits about each other the radii of which are equal to the distance between the threads. Thus relatively considerable distances must be traversed against resistance. The expected result is two threads coiled in matching helices but not intertwined as is the case with “sister” strands in meiosis (see Wilson and Hutcheson 1941). Two strands coiling in separate matrices would be completely independent whether homologous or not (Huskins and Wilson 1938 and Wilson and Hutcheson 1941).

The formation of a true helical minor spiral poses a somewhat more difficult problem. While the compression hypothesis might allow for an irregular one-plane waviness in an heterogeneous thread, it is not easy to see how a three dimensional helix could be formed unless we assume a matrix within a matrix. It appears more likely to us that the minor spiral, if ultimately proven to be a true helix, may be an internally determined “equilibrium” spiral not necessarily resulting in over-all torsion. Variation in the degree of this coil or waviness would provide a satisfactory mechanism for chromonema length change, which would result in the visible coils under suitable conditions.

It is also unlikely that chromosome relational coiling can come about as a direct result of a compression mechanism. The current lack of quantitative data renders any discussion of the probable factors involved somewhat speculative. We are inclined to think that some form of positive torsion may be involved but not with the precision and universality which Darlington and others suppose. There is good reason to believe that the optically single leptotene thread was clearly double in the previous division (Smith 1942). In that case the potential cleavage plane is almost certainly rotated to some degree. As contraction occurs after pairing (and probably chiasma formation) chromatid relational
coiling may be resolved in favor of chromosome relational coiling as a certain amount of tension develops in the contracting threads. This would occur only in those cases where chromatid relational coiling is consistent in direction for complete arms and where homologues are equivalent in direction and, to some extent, in degree of coiling. On this basis, chromosome relational coiling would be sporadic and fortuitous which, so far as available data go, appears to be the case.

4. Conclusions

The properties of readily visible helical and chromatid relational coils do not agree at all with expectation based on coiling produced by internal torsion but do fit expectation from the compression hypothesis. At the same time, such coils as the minor spiral and chromosome intertwining in meiosis appear more likely to involve some degree of torsion or tension internally dictated. We would suggest that chromonema contraction is the result of spatial rearrangement at the molecular level and that such rearrangement under certain specific conditions may give rise to or facilitate the production of minor spirals and chromosome relational coiling. Helical coiling of the type of the major and standard coils as well as chromatid relational coiling are the direct result of differential length change between matrix and chromonema as previously suggested and are only indirectly related to molecular rearrangement. Indeed during the production of these coils any torsion which does exist might well be approaching a minimum. Such a hypothesis appears to fit all available data rather well and to eliminate the major objections of the proponents of the two hypotheses to each other's viewpoints.

References

Sax, K. and K. L. Humphrey 1935. The structure of meiotic chromosomes in micro-


Explanation of Plate IV

Figs. 1 & 2: Interpretative drawing and photomicrograph of a metaphase chromosome, from Allium root tip, indicating spiral direction in sister chromatids.

Fig. 3: Prometaphase from Allium root tip showing internal structure. The chromonemata are helically and the chromatids relationally coiled.

Fig. 4: Pachytene of Podophyllum showing both chiasmata and chromosome relational coiling.

Figs. 5 & 6: Late prophase and early anaphase of the first microspore division of Tradescantia reflexa showing internal structure.