A MICRO DISSECTION OF THE PACHYTENE THREADS OF TRADESCANTIA VIRGINICA L. WITH OBSERVATIONS ON SOME ASPECTS OF MITOSIS.

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PLATES 1 AND 2.

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As a report of progress on some work in support of the note on *Tradescantia virginica* L. published in *Science* (1922) under the title Perigenesis, it seems desirable to issue an abstract of some further studies with Chambers' modification of the Barber pipette. Owing to the pressure of other problems it has been impossible to prepare a detailed account of the large amount of material that has accumulated since it involves many drawings and photographs.

Pollen mother cells of *Tradescantia virginica* L. were teased from the anther sacks and suspended in sugar solution or plant sap as described in my paper published with Professor Chambers (Chambers and Sands, 1922–23). A particular study was made of the pachytene threads as illustrated in Plate 2, Figs. 13, 14, 15. These are evidently referred to by Gates (1924) as having been observed recently in *Œnothera* and no doubt form the basis of his comments. It is to be noted that at this stage the fusion of the paired threads, as assumed by those who adhere to the doctrine of parasynapsis, has supposedly been completed. From the knot in Plate 1, Fig. 1, and Plate 2, Fig. 14, the filament loosens and becomes distributed into the nuclear cavity (Plate 2, Fig. 17), there to undergo segmentation in what has been termed second contraction.[1] (Compare Mottier, 1907, Plate 28, Fig. 38, and Davis, 1911, Plate 71, Fig. 14, with

[1]The use of the term synapsis is avoided. Synizesis might be employed more properly. I should prefer rather to limit synapsis to the phenomena involving the linkage of the chromosomes in a continuous chain in distinction to conjugation of abstricted elements end-to-end so as to cause a reduction of the segments.

Plate 1, Fig. 4 of this paper.) In *Tradescantia* the rods and rings arise directly from this stage. They follow segmentation directly without the appearance of any clear-cut diakinesis stage prior to the inception of the equatorial plate phenomena.

With the dissecting needles it is often possible to unravel this heavy skein (Plate 2, Figs. 14, 15, 16), just prior to the stage shown in Plate 2, Fig. 5. The knot is dissected from the nuclear cavity by cutting away the cellulose membrane together with part of the surrounding cytoplasm so that the skein lies free in the suspension fluid. The partial operation is shown in the photomicrograph, Plate 2, Fig. 13. When the knot is normally about ready to loosen and proceed further with segmentation the filament may often be resolved into a continuous thread with or without constrictions. (See also Davis, 1911, Plate 71, Fig. 14.)

The advent of the constrictions seems to vary in individual cells because some seem to have reached the stage of partial segmentation when still quite closely contracted. Other cells seem to be quite late in reaching this stage and these are the most favorable for manipulation with the needles.

The stages of the formation of the achromatic figure and the events within the filament itself do not always seem to be absolutely synchronized. Professor Wilson (1925, p. 121) notes that, "We may conveniently treat the history of the chromatic and the achromatic figures as if they were separate, though closely parallel processes."

The constrictions mark the boundaries of the chromosomal elements and, since they do not all appear simultaneously, quite variable lengths of the thread may be cut off.

Ultimately the filament is mapped out into somewhat regular sections. This may often occur while it is still somewhat contracted. An inspection of Plate 2, Fig. 13, shows the sausage-like forms these units may assume. Mottier (1907) thought that the rods respliced after segmentation, but the abstrictions observed were, on the contrary, unquestionably incomplete, thus leading to linkages which continue up to and often including metakinesis. (See Plate 2, Fig. 18.) As the knot loosens and distributes itself into the nuclear cavity, certain of the constrictions become complete abstrictions, thus cutting off segments of the filament united in pairs. Many

investigators have referred to these elements as bivalents. In *Tradescantia* Belling (*Genetics*, 1925) recently refers to them as such. Except in the case of supernumerary chromosomes, the writer designates them as tetrads, the reason for which will soon appear, although the tetrad nature may not be evident in their morphology. *Many counts here show that the number of so called pairs correspond to one-half the number of somatic chromosomes* (compare vom Rath, 1892).

If these are theoretically tetrads, a sufficient number of univalents will be present to give each of the four resulting pollen grains its haploid quota and the cell itself would therefore be tetraploid. In some forms tetrads, directly recognizable as such, are found as noted below.

For the purpose of greater clearness, the abstriction which cuts off tetrads will be defined as the *primary abstriction*. It results from a completion of the *primary constriction*. By primary constriction, it is not intended to indicate that this is the first which may appear in the continuous thread. The earlier stages have not yet been followed out by dissection. It is the constriction which, by its completion, leads to the primary abstriction.

Beginning with such a stage as Plate 2, Fig. 14 (fixed material), of Plate 1, Fig. 1 (living material), dissections show the stage given in Plate 1, Fig. 2, may sometimes be resolved into a result such as is shown in Plate 1, Fig. 4, in which the filament is continuous and made up of a medulla with an outer rind,—more clearly seen by *intra vitam* staining, as described by me in 1922 and 1923, and also by Chambers (1914, 1925). The structure here is that of the chromosomes figured in my paper of 1923.

Plate 1, Fig. 5, shows a condition found in many cells where the constrictions seem to be either further advanced or the segments, at some points, are less strongly united. In Plate 1, Fig. 6, primary abstriction has occurred at the points marked (*a*). A *secondary constriction* appears at the point (*b*) producing the familiar bivalent figure of Mottier (1903), and many others. In the case of *Fossombronia* (Farmer, 1895), *Pteridophytes* (Calkins, 1897), *Arisæma* (Atkinson, 1899), *Chiloscyphus* (Florin, 1918), and others, another constriction prior to the first metakinesis appears at (*c*) so that, at this time, a picture of morphologically perfect tetrads is presented.

In *Tradescantia*, constriction (*c*) does not normally begin to appear until about the time of the equatorial plate. Sometimes it may be slightly before or after this. By stretching the so called bivalents with the dissecting apparatus, as shown in the photomicrograph (Fig. 9, of my paper with Professor Chambers—Chambers and Sands, 1922–23), the organization within the chromosomal mass at (*c*) becomes somewhat evident at an earlier stage than normal. Plate 1, Fig. 11, of this paper, is a drawing of that figure. The masses of the univalents in both members are partially outlined *m*, *n*, *o*, *p*. When the masses are released from the needle points they resume the bivalent form shown in Plate 1, Fig. 12.

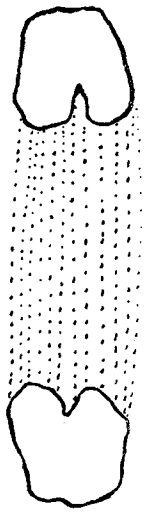
Where closed rings have not been formed, separation on the metaphase spindle of the first division occurs at (*b*) Plate 1, Fig. 6. The constriction here will be referred to as the *secondary constriction and its completion at metaphase, first division, leads to the secondary abstriction.* The constriction at (*c*) will be referred to as the *tertiary constriction.* During the first division, this constriction does not go as far as abstriction, but arrives at this stage in the metaphase of the second division, where univalents, *m*, *n*, Plate 1, Fig. 11 etc., are separated and a return to the haploid condition is reached (meiosis).

The interkinetic nucleus is therefore diploid (*amphikaryon*). If the mother cell nucleus is diploid, quantitative reduction (assuming no longitudinal splits, etc.) would occur on the first division spindle. However, according to my conception, it has become tetraploid by reason of perigenesis (1922); so that the reason for two reduction divisions becomes apparent. Normally, *i.e.* where no supernumeraries or odd chromosomes due to hybridity are present, there are as many tetrads as haploid chromosomes (vom Rath, 1892, in copepods, calls them segments). It is assumed that perigenesis is suppressed during interkinesis. Some investigators, notably Wilson (1912, *Oncopeltus*), find no return to the resting condition in interkinesis. (See also Richards (1917).)

The inception of the tertiary constriction in *Tradescantia* starts during the equatorial plate stage of the first division and progresses through the anaphase. In the late anaphases or early telophases there may still persist a fine thread of achromatic substance connecting the two masses of chromatic material. Each of these masses is assumed to be univalent and has been so considered by most

other authors. One may find the dyads clearly defined in fixed material, but more often, especially when the chromosomes are short, they are fused so closely by the rigors of fixation that they give pictures like Text-fig. 1 (see also Allen, 1905, Plate 8, Fig. 79).

A constriction analogous to the tertiary constriction has not always been observed in the anaphases and telophases of the first division of the pollen grain nucleus which separates the vegetative and the generative nuclei, although in both cases the chromosomes advance to the poles in the form of V's. The conditions shown in Plate 1, Fig. 10, can easily be observed in either the living condition, *i.e.* in plant juice suspensions, or after *intra vitam* staining by weak methylene blue. Aceto-carmine, as outlined by me in 1923, gives good results. In *Tradescantia* the masses *o* and *p*, also *m* and *n*, Plate 1, Fig. 10, appear about equal.



TEXT-FIG. 1
Fixation fusion of the arms. Anaphase dyads of the heterotypic division in *Tradescantia virginica* L. Flemming's strong solution with triple stain.

The free ends *a*, *a*, Plate 1, Fig. 6, may remain far apart to form rods, Plate 1, Fig. 9. With *intra vitam* staining a clear space is usually observed at (*b*), the secondary constriction. *The same free ends may meet and give true rings* (Plate 1, Fig. 7). (Compare vom Rath, 1892, Rückert, 1892.) Under favorable manipulation with the needles at this time the rings may often be impaled through the central opening. The structure of the elements, in cross-section, is still that of an achromatic cylinder, the same as for the continuous filament of Plate 1, Fig. 4.

Where the primary constriction has not been completed, as at a_x Plate 1, Fig. 8, two tetrads are often left united. This results in an octad linkage. If the free ends *a*, *a*, of this figure unite, a larger ring than normal is formed—an octad ring. In this fashion, sextad rings may also occur, especially where odd chromosomes are present.

The separation of rings such as those in Plate 1, Fig. 8, is accomplished in different ways but mostly as figured by Miyake 1905, Plate 5, Fig. 147, and as I shall describe more fully at another time.

During the telophases of the first heterotypic division, the dyads become completely dispersed simultaneously with the reformation of the nuclear membranes. At its completion, the nuclei are not distinguishable from resting nuclei. The second division shows the usual prophase stages found in vegetative divisions, with some minor differences.

To recapitulate, we have started with a continuous thread that can be demonstrated to be such by the technique described above. In my note entitled Perigenesis (1922) it was pointed out that this thread was a chromatically hollow cylinder, in which the chromatin exists only near the periphery and with the structure of the chromosomes as later (1923) described by me. According to my findings, this chromatically hollow structure of the filament can be recognized in the earliest prophase stages even before parasynapsis is supposed to have occurred. This could not be done with material in any way more coagulated than that obtained by aceto-carmine fixation. Lately, this same structure has been figured by Chambers (1925).

The apparently double nature of the strands and granules of Plate 2, Figs. 6*b*, 10, 11*a*, 11*b*, of Chambers' work has been offered as evidence for parasynaptic pairing. The argument would be more convincing but for the fact that the strands are not double but are, in cross-section (Fig. 7*a*), cylinders with a plainly defined medulla. The development of the filament from its earliest observable stage is presented by Professor Chambers in Fig. 7*a* and 7*b*. The finished structure quite closely agrees with that described in my paper published in *Science* (1922). I cannot conceive of any simple means by which so complex a form could arise from a side by side pairing.

It is quite true that fixation of this structure by chrom-osmo-acetic acid will give pictures in optical sections that can be interpreted as double by ignoring the cross-section appearances of spiremes, chromosomes, and telophases.

I therefore look upon the conclusions of Pfitzner, 1882, Müller, 1912, on *Naias marina*, Martens, 1922, on *Paris*, and others, as based on an artifact which from the earliest investigations has lead to conceptions of single longitudinal splits, double longitudinal splits, precocious telophase splits in preparation for the next division, chro-

monemas, development and fusion of internal chromosome vacuoles, chromatic spirals both double and single, and a telophase quadripartite structure developing not only elements that will be separated on the homeotype spindle but at the same time those that will be separated on the spindle of the pollen grain haploid nucleus in forming the vegetative and generative nuclei.

The pairing that *does exist is quite distinct from any of these appearances* and will form the subject of a further paper. Throughout the whole literature of mitosis, the fact that the possible quadripartite structure of the filament (Sands, 1923, Plate 29, Fig. 1), could lead to misinterpretation, has, with but few exceptions, been consistently ignored.

In neither *Tradescantia* nor in *Rhœo,* which has been as fully investigated, was any further evidence for side by side pairing found nor was a longitudinal split apparent. Without the added data from micro dissection it is quite clear from other papers, (Stout, 1912–13, Mottier, 1907, Davis, 1911, Suessenguth, 1921, Gates, 1924, as well as many of the earlier workers) that the relation of the chromosome masses within the spireme is a matter of continuous linkage in a chain.

The process by which this linkage is arrived at, I would term *synapsis* in contradistinction to the reassociation of abstricted elements end-to-end as described by Rückert, 1892, and others. It is seen from this, that, at least for *Tradescantia, division and segregation are everywhere processes of abstriction with subsequent mechanical distribution of the elements.* Where linkage occurs, it is owing to the fact that constrictions may lag, or that abstriction may be suppressed in some cases perhaps permanently.

The data here presented will later be more fully discussed and illustrated. It is obvious that the contentions of Haecker, 1895, Belajeff, 1898, Strasburger, 1900, and others concerning the division and separation of the *idants* cannot apply for *Tradescantia. Tradescantia* does not show a longitudinal split in the prophases and hence the use of the term *equational division* should be avoided, except as it implies the separation of sister chromosomes that have arisen by abstrictions in pairs (two univalents) from a continuous filament.

SUMMARY.

A micro dissection of the pachytene threads of *Tradescantia virginica* L. shows that the relation of the chromosomes is a matter of continuous linkage in a chain and that, undoubtedly, division and segregation are everywhere processes of abstriction with subsequent mechanical distribution of the elements.

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EXPLANATION OF PLATES.

PLATE 1.

Free-hand drawings from the living material of *Tradescantia virginica* L. suspended in *Presssaft* from the plant or in cane-sugar solution.

FIG. 1. Pachytene knot prior to dissection.

FIG. 2. The same as Fig. 1 after cutting away part of the cellulose membrane.

FIG. 3. The knotted thread partially unravelled.

FIG. 4. A continuous spireme resolved from such a stage as Fig. 3.

FIG. 5. Constrictions too far advanced to give such a stage as Fig. 4.

FIG. 6. A bivalent tetrad.

FIG. 7. A tetrad ring.

FIG. 8. An octad ring.

FIG. 9. A tetrad rod.

FIG. 10. Anaphase of the first reduction division showing univalent bodies *m, n, o, p.*

FIG. 11. A drawing of Plate 1, Fig. 9, *J. Gen. Physiol.*, 1922–23, v, 815.

FIG. 12. A drawing of a tetrad such as Fig. 9 of this plate after stretching as in Fig. 11 and releasing again.

Drawings about × 2500.

PLATE 2.

Photomicrographs taken with a Zeiss apochromatic 2 mm. N.A.1.4 oil immersion, compensating ocular No. 8. Bellows at 50 cm. Leitz Lilliput arc, color screens, and panchromatic emulsions. Enlargements × 1600.

The dissection of Fig. 13 was in *Tradescantia Presssaft.* Fig. 14 was an aceto-carmine preparation. Figs. 15, 16, 17, and 18 were from material killed in Flemming's strong solution, etc., etc., triple stained.

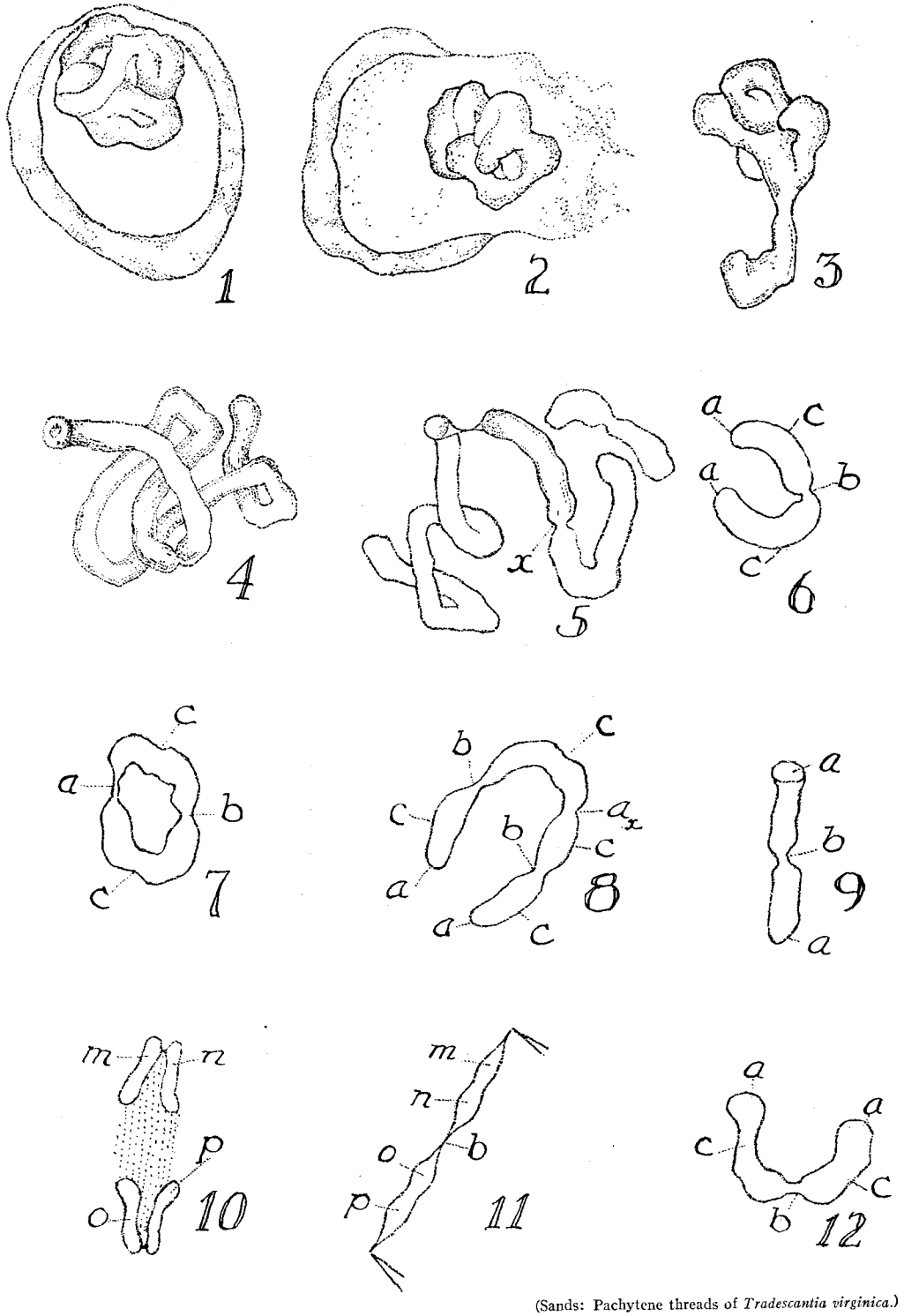
FIG. 13. The removal of the pachytene knot from the nuclear cavity.

FIG. 14. The same stage as Plate 1, Fig. 1.

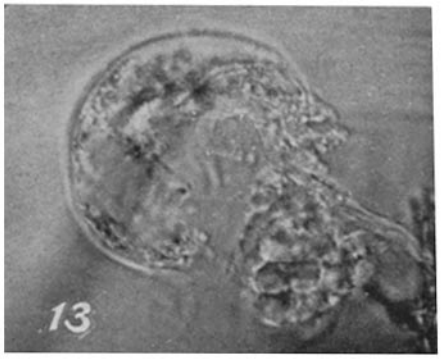
FIG. 15. The opening up of the pachytene knot.

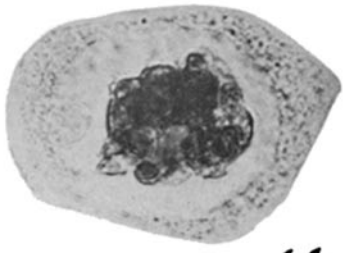
FIG. 17. A later stage showing constrictions, in some instances abstrictions and the beginnings of ring formation.

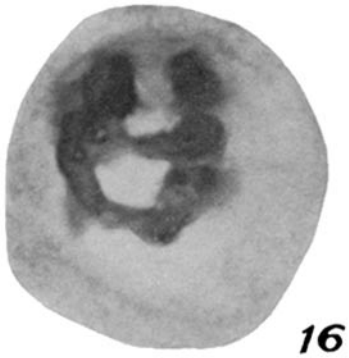
FIG. 18. Incomplete abstriction on the equatorial plate. (Very common in *Rhœo,* an allied genus.)

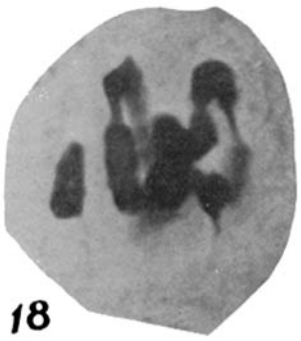


(Sands: Pachytene threads of *Tradescantia virginica*.)









(Sands: Pachytene threads of *Tradescantia virginica.*)