Meiosis in *Tropaeolum majus*, L.

Contributions to Cytology and Genetics from the Departments of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 45

By

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With Plate I

Since STRASBURGER has described in 1880 about the mode of cell division by furrowing in the pollen-mother-cells of *Tropaeolum majus*, L. in his well known work "Zellbildung u. Zelltheilung," we have no further cytological researches about this plant, so I have undertaken a cytological investigation about it, and could confirm his observation, that the tetrads formation takes place by furrowing. At the same time I found some interesting features in heterotypic division, and in regard to chromosome numbers, the brief account of which will be given in the following pages.

MATERIAL AND METHODS

The material was taken in various sizes from young flower buds, fixed with FLEMMING's stronger solution for about 24 hours, and washed one day long. Sections were cut about 14 micra in thickness and stained with HEIDENHAIN's iron-alum haematoxylin for about 48 hours.

OBSERVATIONS

Early prophase. In the very early heterotypic nuclei, karyoplasma takes the stain very weakly, often almost imperceptibly. In a little later stage, however, the chromatin aggregates are observed here and there in the nucleus (Fig. 1).

As the stage advances, chromatin masses are found along the achromatic thread which extends in the nuclear cavity (Figs. 2, 3, 12).

Then the nuclear contents are withdrawn from the periphery.

It indicates the approach of the first contraction. By this time the pollen-mother-cells are loosely separated from each other. Sometimes this separation does not take place until the first contraction.

Synapsis. It seems that the synaptic contraction comes on rather suddenly as other authors described about it in other plants. And it is difficult to trace a full series of intermediate stages between fully developed loose reticulum and the synaptic knot.

In complete synapsis nuclear periphery is quite clear and the threads are aggregated towards the one side of the nucleus so closely that the entire mass seems like a solid ball (Figs. 4, 5, 6). Soon the threads loosen up and the nucleolus lies to one side of the synaptic ball and is partially embraced by it (Fig. 7).

Soon in the loosening-out stage the knot becomes less granular and more fibrillar (Figs. 8, 9, 10).

The loosening out of the univalent spireme from the synaptic knot is very much more gradual than in the previous stage of contraction.

As the nucleus passes into the open spireme, it becomes much more granular and reticulate than in the presynaptic stage. And I have seen at contact points where there is the union of univalent strands chromatin beads.

Gradually the spireme begins somewhat to contract, (Figs. 13-18), decreasing in number of chromatin granules, while increasing in number of chromatin masses, and withdraws from the periphery of the nucleus to one side, and form several loops (Fig. 19), when strands become thick. The second contraction is not so conspicuous as the first. In these figures of second contraction points of junction of chromosomes as well as individual chromomeres are seen rather clearly (Figs. 20, 21). Next happens the segmentation of the spireme, and the segments, suddenly thickening become rod-shaped.

Next appear 4 paired rings, one of which is larger than the others (Figs. 22, 24, 26, 27), and 20 unpaired rods, some of which unite by twos to form bivalent longer rods and are arranged in irregular manner (Figs. 23, 25). Each ring consists of 2 univalent chromosomes united end to end. Thus conjugation seems to have occurred telosyndetically.

Sometimes 4 paired-chromosomes form half rings instead of complete ones, but they are of the transitory form; sooner or later they form perfect rings.

Diakinesis. In a short time the chromosomes shorten in both length and width, and they are arranged themselves side by side in pairs to form gemini; unpaired chromosomes showing the similar behavior (Figs. 28, 29).

In this stage we can hardly distinguish whether several of them were formerly paired rings or not; they are almost in the same size.

Metaphase. Soon after nuclear membrane and nucleolus disappear, and the chromosomes are arranged at the equatorial plate, almost in contact with each other in pairs.

In the metaphase we can easily count the number of chromosomes (Fig. 35). The diploid number of chromosomes in *Tropaeolum majus* (SUTTON's Scarlet Tall) is 14. There is no irregular behavior of chromosomes as often found in hybrid plants. Chromosomes arranged in the equatorial plate gradually proceed to both poles in a regular manner (Figs. 30-32, 34).

Anaphase. As soon as the daughter chromosomes are arranged at the poles they contract into 2 compact masses, which correspond to the stage "tassement polaire" (Figs. 36, 37). Here the anaphase ends and telophase begins.

Telophase and Interkinesis. After closely tightening together for a short time the daughter chromosomes begin to separate themselves, the individual chromosomes becoming visible again.

Soon after there appear nuclear wall and nucleolus (Figs. 38, 39). And chromosomes become drawn out to form irregular figure and lie in the coarse reticulum (Fig. 39). As the reticulum almost fades away granular chromosomes arrange themselves in the periphery of the nuclear cavity (Fig. 40). Gradually the volume of the daughter nucleus becomes larger than ever, and chromosomes and numerous extranuclear nucleoli appear (Fig. 41).

The splitting of chromosomes occurs during interkinesis.

Homoeotypic division. In the homoeotypic division we see that the metaphase is similar to that of the heterotypic division. Two metaphase spindles lie either in one plane or in two planes at an acute angle or at right angle to each other (Figs. 42, 47, 43).

In the daughter nuclei granular chromosomes are arranged themselves very close together (Fig. 47), but soon nuclear membranes and nucleolus arise and chromosomes seem to be disintegrated into

tiny chromatin granules. The 4-nuclei lie almost equidistant from one another in the cell (Figs. 45, 46).

The wall formation of the dividing pollen-mother-cell is brought about by furrowing, and is developed after the 4-nuclei are formed, or immediately after interkinesis (Figs. 44, 48). Fig. 43 shows a case when the first wall formation is almost complete while the daughter cells are still in the metaphase.

In any way the first trace of wall formation consists in slight protuberances, appearing at the periphery of the protoplast, midway between the two nuclei (Fig. 46). They proceed inward (centripetally) rather rapidly and finally divide the cell into 4 spores (Figs. 48, 49, 50).

DISCUSSIONS

Telosyndesis. In the postsynaptic stage I have seen as above stated, there are 4-bivalent ring-shaped chromosomes conjugating telosyndetically, and 20 univalent chromosomes, some of which may unite by twos to form longer bivalent rods in irregular forms. That the hybrid plants, whose paternal and maternal number of chromosomes is different from each other, show often both bivalent and univalent chromosomes in the postsynaptic stage was demonstrated in several cases, e.g. ROSENBERG's *Drosera*, DIGBY's *Primula*, GATES' *Oenothera*, ROSENBERG's *Hieracium*, KIHARA's *Triticum*, BLACKBURN & HARRISON's and TÄCKHOLM's *Rosa*, and YASUI's *Papaver*. Above all BLACKBURN & HARRISON, in their paper on *Rosa* used the existence of the univalent chromosomes in the postsynaptic stage as the criterion between the pure forms and hybrids.

But TISCHLER, GEERTS, and GATES hold the view that univalent chromosomes in this stage of meiotic phase are also found in pure species.

In my present case of *Tropaeolum* too I saw both bivalent and univalent chromosomes in spite of this plant being a fixed form, although there is still no decided proof that this form is not a hybrid. However the fact that all the chromosomes become to be paired at the diakinesis is in favour of the view that this plant is a pure form.

The pollen-tetrads. The tetrads formation takes place by furrow-

ing exclusively and no cell plates have been found, just as is the case with TAHARA's Shasta Daisy (1915). Sometimes after the heterotypic division, cleavage furrows start to form and in the homoeotypic metaphase its development towards the center to form a wall across the diameter of the cell is almost completed as is shown in Fig. 43.

There are differences between the special wall formation of *Tropaeolum* and FARR's *Magnolia* that in the latter case cleavage furrows start to form after the first division, but its development is suspended until the end of the second division, while in the former the wall formation is almost completed in the metaphase of the second division without stopping till the end of homoeotypic division. And at the end of the second division another furrows appear at both ends of newly formed cells, and in the event 4 spores are formed. In the homoeotypic division the 2 metaphase spindles are parallel to each other, and lie sometimes in one plane (Fig. 42), but often in two planes at right angle or at an acute angle to each other (Figs. 43, 47).

In short, the mode of tetrads formation of *Tropaeolum* belongs to the type which is characteristic of Rodophyceae, some monocotyledons and dicotyledons.

Relation between telosyndesis and tetrads formation by furrowing.

In angiosperms it seems that there is a certain coincidence between telosynapsis and tetrads formation by furrowing as is seen in GATE's and CLELAND's *Oenothera*, DENHAM's *Gossypium*, SINOTÔ's *Rumex*, and my *Tropaeolum*.*

But how far this coincidence extends is a question which must be left open until a larger number of cases has been examined.

By the way I may add here that the pairing of the chromosomes in the somatic cells is also demonstrated in *Tropaeolum* as Fig. 52 shows. SUTTON's Salmon Pink, Yellow Tall and Yellow Dwarf show the same behavior of chromosomes, and that in the cortical parenchyma of these SUTTON's forms of *Tropaeolum* there are found amitotic figures, often 3 or more nuclei being present in a cell. Fig. 51 shows an example in the cortical cell of the root of SUTTON's Salmon Pink. Similar observation was made by McLEAN (1914) on several aquatic angiosperms.

* I have also found that *Polygonum Savatieri*, NAKAI and *Beta vulgaris*, L. are additional examples in this respect.

SUMMARY

1. The number of chromosomes in the pollen-mother-cells of *Tropaeolum majus* (SUTTON's Scarlet Tall) is 14.
2. In the postsynaptic phase there are 4 paired chromosomes forming 4 rings telosyndetically and some bivalent chromosomes forming long rods, the other univalent ones forming shorter rods in irregular forms.
3. In the diakinesis both chromosomes, paired and unpaired, somewhat contract and are arranged in pairs.
4. The wall formation in the dividing pollen-mother-cells is centripetal and usually it begins at once when the second division is over and 4 spores are formed. But sometimes it begins in an earlier stage, even at the end of the first division, first dividing the cell horizontally, and after that the second wall being formed crosswise to the newly formed cell wall.
5. The pairing of the chromosomes in the somatic cells is also demonstrated in *Tropaeolum*.
6. In the cortical parenchyma of *Tropaeolum* there are found amitotic figures, often 3 or more nuclei being present in a cell.

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Explanation of Plate I

All figures were drawn at the desk level, with the aid of an ABBE's drawing apparatus with LEITZ's 1/12a and ZEISS' comp. ocul. under the illumination of BAUSCH & LOMB's 6 volts 30 watts microscopical lamp.

- Fig. 1. Very early prophase.
Figs. 2,3,12. Early stages of prophase.
Figs. 4,5,6. First contraction.
Fig. 6. Shows a small granule is attached to nucleolus.
Figs. 7,8,9,10,11. Loosening of the synaptic ball.
Figs. 13-18. The achromatic thread is gradually becoming concentrated and appears to withdraw from the periphery and aggregates to one side.
Fig. 15. Open spireme.
Fig. 19. Second contraction.
Fig. 21. The points of junction of the univalent chromosomes are seen.
Figs. 22-27. Postsynaptic phase, showing 4 chromosome-rings, one of which is a little larger than others.
Figs. 28,29. Diakinesis.
Figs. 30,31. Metaphase.
Figs. 32,37. Anaphase.
Fig. 35. Shows 14 haploid chromosomes.
Figs. 38,39. Telophase.
Fig. 39. Shows chromosomes assuming irregular figure.
Figs. 40,41. Interkinesis.
Fig. 41. Shows the splitting of chromosomes for the next division.
Fig. 42. Metaphase of homoeotypic division with their spindle axes parallel with each other.
Fig. 47. The same with their spindle axes perpendicular with each other.
Fig. 43. The septum is formed a little after interkinesis, while the two mitotic figures are still in metaphase.
Fig. 44. Formation of the cell wall by furrowing after interkinesis.
Fig. 45. Daughter nuclei already formed, but no furrows yet.
Figs. 46,48,49. Furrowing proceeds towards the center of the mother cell.
Fig. 50. Tetrad is formed within the mother-cell-wall.
Fig. 51. A cortical parenchyma cell of the root of Sutton's Salmon Pink showing 3 amitotic nuclei.
Fig. 52. A root tip cell of SUTTON's Scarlet Tall showing 28 somatic chromosomes in pairs.
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