

Citation:

Sypkens, B., On the nuclear division of *Fritillaria imperialis* L, in:
KNAW, Proceedings, 7, 1904-1905, Amsterdam, 1905, pp. 412-419

Botany. - Prof. J. W. MOLL presents the thesis for the doctorate of Mr. B. SYPKENS. "*On the nuclear division of Fritillaria imperialis L.*", and gives a summary of the results.

(Communicated in the meeting of October 29, 1904.)

The subject of this investigation is especially the nuclear division in the embryo-sac of *Fritillaria*, formerly a favourite material for investigations on the subject of nuclear division.

Mr. SYPKENS studied the free nuclear divisions in the parietal layer of protoplasm as well as the nuclear divisions in the first layer of endosperm-cells which are directly followed by tangential cellular divisions. Besides some observations were made on the nuclei in the ovules of *Tulipa* and in the growing-point of the root of *Vicia Faba*.

All the material was fixed by means of the strong chromo-aceto-osmic acid of FLEMMING. It was for the greater part imbedded in paraffin in various ways and was examined in series of sections of 2 to 4 μ thickness, stained with gentian violet. Some observations were also made by means of the method introduced by VAN WISSELINGH, in which the nuclei are dissolved in chromic acid of about 50%. These two methods supplement each other; the chromic acid method is to be preferred for observations about the chromatic parts, sections give more information about the nuclear spindle. But in this investigation the excellence of both methods was again proved as compared with the observation of the nucleus as a whole, which in many cases renders it impossible to form an accurate idea about its internal structure.

I will briefly mention the chief results obtained by Mr. SYPKENS for the various stages of nuclear division.

The resting nucleus was studied by means of sections and of chromic acid and the results so obtained were in the main a complete confirmation of the results published by VAN WISSELINGH and by GRÉGOIRE and his co-workers WYGAERTS and BERGHS. The framework of the resting nucleus consists of numerous larger and smaller lumps of chromatin, connected by fine threads so that an anastomosing network is formed. There is no reason for assuming in this network the existence of two constituents, chromatin and linin; the chromic acid method as well as coloured nuclear sections show the contrary, if only partial washing out of the stain is prevented, as Mr. SYPKENS did. Those who wish to maintain the assertion about the existence of linin-connections will have to bring forth new and valid proofs.

Also for the nuclei of the integuments and nucellus of *Fritillaria* and of the ovules of *Tulipa* the same results were obtained.

Concerning the individuality of the chromosomes VAN WISSELINGH has shown that it exists in the spirema, since at that stage a continuous thread is never found. But his further observations as well as those of GRÉGOIRE and WYGAERTS indicate that probably, even in the resting stage, this individuality never entirely disappears. Mr. SYPKENS was led to the same conviction by his observations about the formation of the spireme and of daughter-nuclei from the daughter-spiremes. He speaks of a "centralisation and decentralisation of a number of chromatine masses, which in certain stages form as many chromosomes."

About the behaviour of the chromosomes during the process of division little that was new could be found in this investigation for the reason mentioned. The number of chromosomes was fixed at about 60, but in certain nuclei it decidedly is much smaller. Neither is the shape of the chromosomes constant; in the same nucleus U-shaped, as well as V- and J-shaped ones could be found.

The study of the nuclear spindle on the other hand gave important results, not so much about the formation of the spindle as about its further history and the part played by it in cellular division.

The formation of the spindle could be followed in details. Round the free nuclei in the parietal layer of protoplasm of the embryo-sac granular protoplasm occurs with many very small adventitious vacuoles, round the nuclei of the first endosperm-cells also protoplasm with several small vacuoles. Now, when the nuclei begin to divide and the nuclear membranes are dissolved, the surrounding protoplasm penetrates into the nuclear space, at first without many vacuoles, and forms at the interior the spindle-threads, which at first consist of coherent granules and later become smoother. They gradually assume parallel directions and are connected to a bundle without strongly converging towards its poles. The nuclei are then in the spireme-stage. Later, in the aster-stage, besides the threads already mentioned, others are formed in exactly the same way, which grow thicker and only proceed from the poles to the equator, where they are attached to the chromosomes, which have been formed in the mean time. They are found not only at the circumference of the spindle, but also in the interior part of all the longitudinal sections of a nucleus. STRASBURGER has called the former sort of threads, running from pole to pole, "Stutzfasern", the shorter and thicker ones "Zugfasern".

Now metakinesis follows and in the dyaster-stage a separation of the two sorts of spindle-threads has taken place. The shorter

and thicker ones have much contracted and form at both poles, adjacent to the daughter-nuclei, two small caps which soon disappear in the protoplasm. The long threads on the other hand remain between the daughter-nuclei, extending from one to the other and hence are often called connecting threads. They occur in numbers from 200 to 300 and cross-sections show that they form a massive bundle lying free in the surrounding protoplasm, which can freely penetrate between them.

Hence Mr. SYPKENS arrives at the conclusion that the nuclear spindle is entirely formed from the cytoplasm within the nuclear space and so agrees with what has been found by most other investigators and on main points also with the results obtained by STRASBURGER and HEUSER for the nuclei of *Fritillaria*.

Now with regard to the part played by the nuclear spindle in cell-division zoologists and botanists have divergent views. Concerning animal cells the general opinion is that the nuclear spindle is dissolved in the cytoplasm after the nuclear division has been completed and takes no active part in cell-division, the cell subsequently dividing by constriction. Botanists on the other hand, attach great importance to the spindle in cell-division and especially in the formation of the wall. Their generally accepted representation is that the above-mentioned connecting threads of the spindle grow thicker in the equatorial plane and form so-called dermatosomes. By fusion of the dermatosomes the so-called cell-plate is then formed, which subsequently participates in some way or other in the formation of the new cell-wall between the nuclei. STRASBURGER is one of the chief representatives of this much spread conception.

It is a consequence of the fact that the study of this phenomenon has for the greater part taken place with nuclei that were seen from the outside. By means of his sections Mr. SYPKENS was enabled to prove that, for the objects studied by him, the opinion now prevailing in botany is incorrect and that, at any rate as far as the behaviour of the nuclear spindle is concerned, the phenomena have great resemblance with those of animal cells.

In describing the later phases of the nuclear spindle it is desirable to distinguish three different cases of nuclear division. In the first place we have the free nuclear divisions in the parietal layer of protoplasm of the embryo-sac of *Fritillaria*, which will be followed by still other nuclear divisions before there is question of cell-divisions. Here in the beginning a system of connecting threads between the daughter-nuclei appears, as in all other cases, but this soon becomes narrower at the equator and so assumes the shape of an hour-glass and is then

absorbed in the protoplasm and disappears. So this case needs no further consideration.

The second case regards the parietal layer of protoplasm of the embryo-sac, which has already become partly divided into cells. Now when here also free nuclear divisions take place, the nuclear spindle, consisting of connecting threads, behaves at first in exactly the same manner as in tissue-cells in which the cell-division follows immediately: the system of connecting threads swells laterally and forms a so-called nuclear barrel. After this, however, the spindle here is also lost in the protoplasm and not until later one sees successive divisions take place between these nuclei, progressing regularly from that part of the parietal layer of protoplasm that is already divided into cells, so that finally a complete pavement of endosperm-cells is formed from the protoplasm. This description renders the existence of a connection between the nuclear spindle and cell-division not very probable.

The most important case is the third, in which the just-mentioned endosperm-layer divides into two layers of cells by tangentially directed walls. Here the nuclear divisions are immediately followed by cell-divisions, in the same way as in the formation of various sorts of tissues.

Hence this case, as was proved by comparative observations, must be considered as completely analogous with what happens in the cells of the growing-point of the roots of *Vicia Faba*.

From Mr. SYPKENS' sections it appears that in the two latter cases the connecting threads soon cease to deserve that name, as their extremities are not attached to the daughter-nuclei but end freely in the protoplasm. In *Vicia Faba* moreover, the equatorial parts are soon dissolved so that the system of connecting threads falls asunder into two halves.

Meanwhile the protoplasm round the nuclei of the parietal layer of protoplasm penetrates with its small adventitious vacuoles into the space between the daughter-nuclei where the massive complex of connecting threads is found. These threads are consequently forced asunder towards the circumference and thereby united to spindle-shaped bundles, which lie free in the protoplasm; they form what is usually called the nuclear barrel. The result is that the two daughter-nuclei are at last separated from each other by the same granular protoplasm, which also surrounds them and in which also the remains of the connecting threads are found. The spindle-shaped complexes, formed from these, are united to a barrel-shaped, equatorially swollen, cylindrical mantle, which, if the nuclei are only observed from the outside, still seems to join them, although in reality this is no

longer the case by any means. On the contrary, the remains of the connecting threads gradually disappear as if they were dissolved in the protoplasm and this process has long been completed when the cell-walls successively appear between these nuclei also.

Also in the divisions of the endosperm-cells of *Fritillaria* and in the root-tip of *Vicia Faba* mainly the same occurrences take place although there are some points of difference to which I shall refer presently, and although the formation of the cell-wall follows sooner here.

How this wall-formation takes place has for the present not been investigated by Mr. SYPKENS, but that it stands in no relation to the nuclear spindle or to a cell-plate formed by it, is pretty clear from what precedes. A cell-plate in the sense of botanical authors does not even occur.

Although the opinion, so generally spread in botany, that in many cases the formation of cell-walls is dependent on nuclear spindles, may have a certain probability when we only think of the cross-divisions of the cells of growing-points and suchlike, it lacks, generally speaking, every foundation. For any one knows that the formation of cell-walls can in many cases have nothing to do with a nuclear spindle. Not to mention all possible cases of thickening of the cell-wall which do not correspond to the formation of a primary membrane, I will only mention zoospores which, after having come to rest, form a wall; plasmolysed protoplasts of *Spirogyra* and other Algae which cover themselves with a new cell-wall; *Caulerpa* and other Coeloblasts, the protoplasm of which after a lesion produces a new wall-piece.

But also in other cases, which resemble more the cell-divisions in growing-points, it is often easy to show how newly-formed cell-walls cannot possibly have been formed in the nuclear spindle. I mention the antipodal cells, which so frequently are formed projecting inwardly in the embryo-sac connected only for a small part of their surface with the cell-wall of the embryo-sac; in any case no more than a small part of the free wall-surface can have been formed here in a nuclear spindle. A corresponding case is that of the U-shaped walls in the epidermal cells of the leaves of ferns, by which the mother-cells of stomata are formed. More clearly still one sees the same thing in the formation of the stomata of *Anemina fraxinifolia*: the stomata lie in the middle of an epidermal cell of the leaf and the nucleus of this cell is still pressed against the stoma. A nuclear division has taken place here before the stoma-mother-cell was formed in the epidermal cell, and between the two cells so formed there certainly was a spindle at first. But in the subsequent cell-division a

cylindrical wall was formed at a certain distance round one of the nuclei, which consequently could for a small part only have been formed in the spindle. Finally we have the formation of the first pavement of endosperm-cells from the parietal layer of protoplasm of the embryo-sac as well in *Fritillaria* as in many other plants. When the number of nuclei of this layer of protoplasm has very greatly increased, separations between the nuclei arise, so that a layer of flat, pentagonal or hexagonal cells is formed, which at last are separated from each other by cell-walls. These cell-walls are formed at a period when of the originally present nuclear spindles no trace is left.

In relation with these facts the result of Mr. SYPKENS about the negative part played by the nuclear spindle in cell-division cannot surprise us and it even gains in inner probability by them. This result also shows the way to a more profound study of the phenomena of cell-division and wall-formation in the vegetable kingdom. The cell-divisions in growing-points, in the above-mentioned epidermal cells of ferns, also in the parietal layer of protoplasm of the embryo-sac, must now be more closely investigated, preferably by the method applied by Mr. SYPKENS, and important results may be expected of this investigation. Also the study of living, dividing cells, in the same sense as was formerly done by TREUB¹⁾ deserves again our attention in this respect.

It is by no means impossible that by such investigations the conception of cell-division in plants will come still nearer to that of the same phenomenon in animals than is the case at present.

From all that precedes it appears that the nuclear spindle is formed entirely from the cytoplasm and returns to it. Besides, all investigators agree that in nuclear division the nuclear membrane and the nucleoli are dissolved and later are formed anew in the daughter-nuclei. An uninterrupted individual position with regard to the cytoplasm is consequently, among all the parts of the nucleus, occupied by the chromosomes alone, only here there is question of a hereditary organisation.

The opinion of some authors that the nucleus during the whole process of division would form an isolated whole with respect to the cytoplasm and that at first there would be a sort of vesicle, joining the daughter-cells and separating the spindle from the cytoplasm, must consequently be abandoned.

In relation with this I may briefly point out the complete agree-

¹⁾ M. TREUB, Quelques recherches sur le rôle du noyau dans la division des cellules végétales. Publié par l'Académie Roy. Néerl. des Sciences. 1878.

ment between the results of Mr. SYPKENS and the theory of DE VRIES and WENT, which looks upon the vacuoles as hereditary organs of the protoplast. If the nucleus were, during division, an isolated whole, the question about the origin of the vacuoles, present inside the spindle, would perhaps give some difficulty. But we saw, how the observations of Mr. SYPKENS prove that we have here ordinary vacuoles, already present in the granular protoplasm and which are shoved in between the spindle-threads from the outside with the protoplasm.

Yet it will be desirable to give some nearer information about this process, since two somewhat divergent cases occur and here again a distinction must be made between the nuclear divisions in the parietal layer of protoplasm of the embryo-sac and those in the first endosperm-layer or in the meristem of the roots of *Vicia*.

In the latter cases, in which ordinary division of tissue-cells takes place, Mr. SYPKENS observed what follows. In these cells there are a number of vacuoles, which are about equivalent and lie round the nucleus in the granular protoplasm. After nuclear division this protoplasm with its relatively large vacuoles, penetrates into the spindle between the connecting-threads, as we saw above. This penetration here occurs as well in the equator as more in the neighbourhood of the daughter-nuclei. Hence it is the ordinary vacuoles of the mother-cell, which shove in between the daughter-nuclei with the protoplasm in which they lie. Later, when the connecting-threads have been dissolved and cell-division takes place, these vacuoles, as well as those which did not penetrate into the spindle, are divided equally between the two daughter-cells. So the question is here very simple and in complete accordance with what VAN WISSELINGH found in *Spirogyra*. Only in this latter case the mother-cell has not several equivalent vacuoles but a single large one which penetrates laterally into the nuclear spindle.

Somewhat different are the circumstances in the divisions of the parietal layer of protoplasm of the embryo-sac. This cell not only contains many nuclei but has also a somewhat different structure with regard to its vacuoles. It has namely one single large vacuole, filling the middle part of the cell, but besides in the parietal layer of protoplasm a great number of very small adventitious vacuoles, which were very conspicuous in the preparations of Mr. SYPKENS, stained without washing out of the stain. Now, after nuclear division, the granular protoplasm with its many adventitious vacuoles penetrates between the daughter-nuclei and the free extremities of the connecting threads. From there it penetrates further towards

the equator between the connecting threads. Hence the daughter-nuclei are finally separated from each other by granular protoplasm with adventitious vacuoles of the embryo-sac. Now, when later the parietal layer of protoplasm divides into cells, the large embryo-sac vacuole does not partake in this process, but each newly formed endosperm-cell is provided with a certain number of adventitious vacuoles.

So there is a certain antithesis here with what happens in ordinary cell-divisions in young cells, but with the vacuole theory of DE VRIES and WENT this process also is in complete harmony, for WENT has shown that small adventitious vacuoles can occur in large numbers in all sorts of ordinary cells and can in all respects be compared with the large vacuole, from which they can also be produced by division. I should not be surprised if further investigation showed that their occurrence is much more general still than is now supposed.

The case met with in the embryo-sac of *Fritillaria* and many other plants stands by no means isolated, and is also met with in the division of other multinuclear cells. WENT mentions some cases of this kind in his investigation about the vacuoles of Algae. (*Chaetomorpha aerea*, *Acetabularia mediterranea*, *Codium tomentosum*).

I had an opportunity personally to observe a similar case of division in the formation of asexual zoospores in the cells of *Hydrodictyon utriculatum*. While the zoospores, which had been formed by division of the parietal layer of protoplasm, were partly in motion and partly had already arranged themselves to a network, all this inside the wall of the large mother-cell, I saw the middle part of this cell occupied by three great tonoplast vesicles, having their origin in the great central vacuole of the cell and which, upon being heated under the microscope, first shrank and then burst. Hence here, no more than in the embryo-sac of *Fritillaria*, the great central vacuole took part in the formation of new cells. That the zoospores were provided with very small vacuoles, present in the granular protoplasm, cannot be doubted according to the above-mentioned investigations of WENT. I also observed them very distinctly in the cells of the young nets very soon after their formation.

Finally it requires to be mentioned that the doctoral dissertation of Mr. SYPKENS will soon appear in a German translation in the second Part of Volume I of the *Recueil des travaux botaniques Neerlandais*.