The Development of the Pollinium and Sperm-Cells in *Asclepias Cornuti*, Decaisne.

BY

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With Plate VII.

INTRODUCTION.

THE flower of *Asclepias* has always been of interest to botanists. It was studied as early as 1763 by Adanson, and later by Gleichen (1779) and by Sprengel (1793)¹. The mode of occurrence of the pollen has been especially interesting. Loew (1895) mentions a paper by Kölreuter in which the 'hangenden Beutelchen,' later known as pollinia, are described as the 'male genitals' of the plant, and these organs have also been similarly described by Baron Jacquin (1811).

In 1831 Brown, who separated (1867) the Asclepiadeae as a Natural Order of plants from the Apocynaceae of Jussieu, made the first serious study of their pollinia. His first paper on this subject appeared in 1809, but he failed to observe the grains of pollen, and thought that the pollinium consisted of one individual cavity filled with minute granular matter mixed with an oily fluid. In 1831 Bauer, an artist, offered to Brown for publication drawings of several Asclepiadeae, made in

¹ Referred to by Brown, 1833.

1805, and representing 'the internal structure of the pollen-mass as cellular; each cell in the flower-bud just before expansion being filled with a grain of pollen, . . . while in the expanded flower this grain is exhibited as shrivelled, having discharged its contents, which consist of a mixture of an oily fluid and minute granules¹.'

Link considered the pollen-mass as 'composed either of a cellular tissue, or manifestly of grains of pollen: the former part of the description being no doubt meant to apply to true Asclepiadeae, the latter to the Periploceae².'

In 1827 Treviranus described the pollen-mass of *A. curassavica* as 'filled with compressed, nearly round but obtusely angular, colourless, simple grains, containing minute granules; the pressure of the external grains, or those in contact with the general covering, giving it the appearance of being cellular².'

Ehrenberg³ (1829) describes the pollen-mass as 'consisting of a proper membrane bursting in a regular manner, the cavity being not cellular but filled with grains of pollen, each grain having a cauda or cylindrical tube often of great length, and all the tubes being directed towards the point or line of dehiscence.' This appendage he considers analogous to the 'boyau' of Amici (1824) and Brongniart (1827), differing, however, in its forming an essential part of the grain in Asclepiadeae; whereas in other families the application of an external stimulus is necessary for its production. Later, Ehrenberg³ (1831) believes that the grains of pollen are in their earlier stages without the 'boyaux,' which make their appearance subsequently at the period of impregnation.

In 1833, as a result of further researches on the Asclepiadeae, Brown describes the pollen-mass in several species of *Asclepias*, particularly in *A. phytolaccoides* and *A. curassavica*, the figures being drawn by Bauer. According to the description and figures, the pollen-mass consists of cells disposed in three series parallel to its sides, the middle series being often more

¹ Brown (1831), p. 719.

² Brown, loc. cit., p. 720.

³ Quoted by Brown (1831).

or less interrupted. The membrane of the pollen-grain is described¹ as made up of two united coats, and the cavity filled with spherical granules of nearly uniform size. These granules were not often found in the pollen-tubes, nor were the tubes branched or jointed. The figures accompanying this paper are excellent. Brown, without doubt, considers the cells of the pollinium as true pollen-grains.

In 1840 Jussieu figured the pollinia with pollen-tubes projecting, and considered the pollinia as masses of pollen-grains. Schleiden (1849) also considers the pollinia as masses of pollen, clothed with a special membrane.

In his work on the Microscope (1853), Schacht gives directions for the microscopic study of the flower of *A. syriaca*, and describes (Chapter 4) and figures correctly for the first time (Fig. 15) the pollinium mother-cells for this plant. I find no mention of Schacht's work by later writers on this subject. Schacht, however, derives all of the pollen-grains of a single anther-locule from a single 'primary mother-cell,' which he figures (Fig. 21 a)². Engler (1876)³ states that this is not true for *Asclepias*, though it is the mode of development in *Albizzia lophantha*, Benth. The writings of Sprengel (1793) and others I will only mention, as they are concerned with fertilization in the old sense of the term, i. e. pollination⁴.

In 1886 appeared Wille's paper on the development of the pollen-grains in Angiosperms. The first part of this paper deals with the mode of formation of the membrane or coats of pollen-grains. In the Orchidaceae he traces 'a successively retrograde developmental process.

1. Free pollen-grains in *Cypripedium*.

2. Free tetrads with a new membranous structure on the exterior in *Epipactis*.

¹ Loc. cit., p. 724.

² The same writer in his later paper (1860) refers to the cells of the pollinium as pollen-grains.

³ p. 298.

⁴ Meyen (1838), Delpino (1865), Hildebrand (1866), Robertson (1886-7), and Ule (1897).

3. Tetrads held together by the middle lamellae, which have been dissolved into mucilage (*Schleim*) without any special membrane structure for the individual pollen-grains in *Orchis*. It is a step further in the same direction when the middle lamellae between the tetrads remain unaltered. Whether this results in some Orchidaceae I know not, still one finds such a condition in several Mimosae (Rosanoff) and Acaciae (Engler), e.g. *Acacia pulchella*. In Asclepiadaceae (*A. spec.*) the reduction has proceeded still further, for here I have not succeeded in finding a single tetrad division, but the whole mass of apparently undivided pollen-mother-cells were surrounded by an exine-like membrane¹.

Strasburger (1889), referring to Wille's paper, also states that the pollen-mother-cell of *Asclepias* never divides. In *A. syriaca*, he says, one finds the single locule of each half of the anther filled with a row of large, radially oriented cells, rich in content, the primary mother-cells (*Urmutterzellen*) of the pollen. These cells afterwards divide transversely, the longest lying in the middle of the locule, always dividing into four cells, those adjacent to its two walls into fewer cells, in some cases not at all².

Wille, then, seems to have been the first to suggest this interpretation of the structure of the pollinium in *Asclepias*. Vines (1895) gives the same idea, stating that the pollen-mother-cells 'develop directly, without division, into a cell of the pollinium, each such cell being, at any rate physiologically, equivalent to a microspore.'

Later, Strasburger (1900) confirms Raciborski's (1897) account of centrosome-like bodies in the pollen-mother-cell of *Asclepias*, but from the context it is not clear whether he there refers to the individual cells of the pollinium, or to the cell that gives rise to them, and, from the state of our knowledge on the subject at the time Raciborski wrote, we cannot

¹ 'Hier ist es mir nicht einmal gelungen, irgend eine Tetradentheilung zu finden, sondern ganze Massen scheinbar ungetheilte Pollenmutterzellen werden hier von einer eigenartigen Membran umgeben.' Wille (1886), p. 41.

² 'Diese Zellen entsprechen den Pollenmutterzellen anderer Objecte, theilen sich aber nicht mehr.' Strasburger (1889), p. 80.

be sure to just what cells he refers as pollen-mother-cells. From his comparison of the large size of the cells relative to their respective nuclei, it seems as if he must refer to the cells which give rise to the individual cells of the pollinium, for this comparison does not apply very well to any of the other cells of the pollinium. Juel (1900) says that the manner of tetrad division in Asclepiadaceae is still a question.

Neither Juel nor Strasburger (1900) mention Stevens's (1898) paper, nor is any reference made in the latter to Wille's work or to Strasburger's statement. Stevens figures and describes in part the division of the cells from which the pollinium units originate, and calls them pollen-mother-cells.

The general appearance and structure of the pollinium suggest that the cells which compose it are pollen-mother-cells rather than pollen-grains, and this, doubtless, lent ready acceptance to Wille's interpretation of them. The development of these cells, four in a row, from the large primary cells, accompanied by a reduction of the number of chromosomes, as described by Stevens¹, suggests that the cells of the pollinium are pollen-grains. If this is the correct interpretation of them, the arrangement of the cells in a row, rather than at the corners of a tetrahedron, differs, so far as our present knowledge goes, from the mode of formation of pollen in all other plants.

If these cells which develop the fertilization (pollen) tube are pollen-mother-cells, we would expect to find the subsequent nuclear phenomena and the number of cell-divisions to be different from those in the development of the nuclei and sperm-cells in pollen-grains, that is, that there would be a shortening of the process from the mature archesporial cell to the sperm-cells if the microspore were cut out, which would be the case if Wille's interpretation proved to be correct. This has already been suggested by Atkinson (1901). It was for the purpose of determining the course of events in the development of these cells and of the pollen-tube, and the

¹ Loc. cit., p. 81.

nuclear phenomena in the formation of the sperm-cells, that this study was undertaken, for it is clear that the proper interpretation of the structures depends upon a complete knowledge of these phenomena.

METHODS.

Asclepias cornuti was chosen for the basis for the work, but comparisons were made with *A. phytolaccoides*, *A. incarnata*, *A. quadrifolia*, and *A. tuberosa*. The material was collected at frequent intervals during the months of June, July, and August in the summers of 1899 and 1900. Some of it was put up in the field and the rest in the laboratory at different times during the day, from 7 a.m. to 7 p.m. After the removal of the perianth, the buds were halved to ensure more rapid penetration, and placed in Flemming's chromosmic-acetic acid solution for twenty-four hours. The material was then washed in water for twelve hours, dehydrated, bleached, infiltrated, and imbedded in paraffine in the usual manner. The sections were cut of various thicknesses, but those thirteen and one-half microns thick proved best for study. Some of the slides were stained with iron-alum haematoxylin, but the safranin-gentian-violet-orange combination gave the better results.

In order to facilitate the handling of the small pollinia they were placed in tiny bags of cheese-cloth, closed at the top by a small piece of copper wire, one end of which was allowed to project as a handle. The pollinia were placed in these bags after dehydrating.

Pollinia were germinated in a 5 per cent. cane-sugar solution and placed in the Flemming at intervals of from five to thirty minutes during germination. In addition to this, pollinia were examined that had germinated on slices of sugar-beet kept moist in a Petri dish, and also those that had germinated on the stigma. Their behaviour seemed to be the same under each of the above conditions. A rather vigorous circulation of protoplasm was noticeable in freshly germinated pollen-tubes.

THE ARCHESPORIUM.

Sections through young buds about 3 mm. in diameter showed the five young anthers around the central axis of the flower, each having two well-defined oval areas containing a varying number of large, long cells, arranged radially, and having comparatively large nuclei (Pl. VII, Fig. 1). These areas occupy the position of the pollen-masses in older buds, and so, evidently, locate the locules.

Much younger buds were then sectioned until a stage was reached where there was no marked indication of differentiation of cells in the anther. Such a stage is shown in Fig. 2, where cell-division is taking place in various directions throughout the tissue. Soon, however, these divisions cease, and a row of sub-epidermal cells in each end of the anther on the proximal side appear larger and richer in protoplasmic content. They are usually straight, but at a later age some of them have a slightly sigmoid form. Division in these cells is always in periclinal planes, cutting off a sub-epidermal cell from an inner cell, which is later seen to give rise to the cells of the pollinium, and which will be referred to as the primary pollinium-cell (Fig. 3). The division is shown complete in Fig. 4. This process varies from that described by Schacht (1853), who derived all the cells of a single pollinium from one primary mother-cell.

THE TAPETUM.

The sub-epidermal cells undergo many divisions in different planes, as shown in Fig. 5. Parallel with these changes the other tissue-cells immediately surrounding the primary pollinium-cells become altered, until finally a well-defined tapetal layer surrounds the primary pollinium-cells (Fig. 7). It is thus seen that in *Asclepias* the tapetum is a morphological composite, as described by Coulter (1898) for *Ranunculus*, by Caldwell (1899) for *Lemna minor*, and by Fulmer (1899) for *Hemerocallis fulva*. Frequently some of the primary pollinium-cells appear to disintegrate and function

as tapetal cells, as described by Caldwell (1899). The multiplication of nuclei characteristic of tapetal cells takes place in this layer as shown in Fig. 7. After the pollinium becomes mature, the cells of the tapetum disintegrate.

THE PRIMARY POLLINIUM-CELLS.

The young primary pollinium-cells now pass through an extended period of growth, elongating greatly until they are from four to six times as long as broad. They are oriented with the long axis radial as described by De Mirbel¹ (1833) for *Cucurbita*, by Juel (1900) for *Carex acuta*, and as first described by Schacht (1853) and later by Stevens (1898) for *Asclepias*. The nuclei increase in size and the nucleolus, which often occupies over one-half of the nuclear cavity, stains deeply with gentian-violet.

About this time also, from one to two little wart-like bodies appear at the edge of the nucleolus, similar to those described and figured by Wiegand (1899) for *Potamogeton*, and interpreted by him as probably the true nucleoli. I could not make out any constant difference in the staining reaction of these little bodies and the large one to which they are adjacent, as described by him. In Fig. 9 two of these bodies are shown for each nucleolus, and in Fig. 10 there is only one, while in Fig. 11, which is a later stage, there are none. Figs. 13 and 14, which represent sections through the nucleolar structure of the pollen-mother-cell, suggest that in *Asclepias*, at least, these wart-like bodies are a stage in the fragmentation of the nucleolus. Fig. 13 suggests that five bodies have been given off from the large one, and, if this is the correct interpretation of the condition there figured, it is probable that Figs. 13 and 14 represent successive stages in this division. From the later condition shown in Figs. 12 and 16, it seems probable that this fragmentation of the nucleolus does not always take place. Associated with this phenomenon of growth and fragmentation, is a more or less vacuolated

¹ Quoted by Nägeli (1842), p. 7.

condition of the nucleolus as shown in Figs. 10 and 12-16. When there is no fragmentation there are usually no vacuoles present, though this is not always the case.

Fragmentation of the nucleolus has been described by Farmer (1893) for *Lilium Martagon*, and (1895) for *Pellia epiphylla*, where the particles into which it breaks up are found usually on young chromosomes or close to them. Farmer suggests that this proximity is due to 'mechanical' causes. From the staining reactions in *Asclepias*, the suggestion is very strong that it functions as a reservoir for chromatin or for some allied substance, as Farmer (1895) and Pfitzner have suggested.

Miss Sargent (1896), in her studies on *Lilium Martagon*, thinks that the nucleolar substance serves as material for the spindle-fibres, or at least to solder them to the chromosomes. This may be its function in *Asclepias*, as the spindle-fibres seem to be of nuclear origin. Other facts, however, argue against this interpretation.

Zimmermann (1893) believes that the nucleoli are thrust out into the cytoplasm, and then in the metaphase taken up again into the daughter-nuclei, '*omnis nucleolus e nucleolo.*' But if this were true in *Asclepias*, then it becomes more difficult than ever to explain the loss of nucleolar substance, as such, after the first division of the primary pollinium-cell, for Stevens (1898), by careful measurements, has shown that the volume of the nucleoli of the daughter-nuclei of the first division is only about one-tenth of the volume of the nucleoli of the primary pollinium-cell nucleus.

After the pollinium-cells are formed they contain prominent rod-shaped bodies which will be referred to later. These bodies stain very deeply with the gentian-violet, and it is possible that this fact may be associated with the disappearance of the nucleolar substance, as such. Stevens¹ argues for the nutritive function of the nucleolus, but thinks that its substance goes to form 'the kinoplasmic spindle and the cell-plate.' On this supposition, however, large nucleoli would be

¹ Loc. cit., p. 83.

as necessary an antecedent to the second division as to the first. If the interpretation suggested above should prove to be correct for *Asclepias*, it could not be considered as the function of the nucleolus in all plants, for the nucleolus is sometimes present after the spindle-fibres are formed¹.

The mature primary pollinium-cell is shown in Fig. 10. In this stage the nucleolus stains very slightly, if at all, with the gentian-violet, while the nuclear reticulum takes a deep blue colour. There is a well-defined nucleolar membrane, and the slightly vacuolated cytoplasm contains many granules evenly distributed throughout and stained with the gentian. These granules first appear at a much earlier stage (Fig. 9), but disappear as division approaches.

THE FIRST DIVISION OF THE PRIMARY POLLINIUM-CELL.

After attaining the maximum size, the primary pollinium-cell passes a comparatively long period during which it undergoes no visible changes. Stevens refers to this as a period of rest. The first indication of approaching nuclear division is the contracted condition of the linin network to one side of the nucleolus, first described by Moore (1894) and later by others, and known as synapsis. The network does not appear to be wrapped about the nucleolus either in whole or in part during this stage. Immediately preceding this condition, the granules on the linin network are more prominent and stain more deeply with the gentian-violet, while the nucleolus takes the gentian only slightly, if at all. During synapsis the network does not take the gentian, while the nucleolus stains a deep blue. There is no reason for considering synapsis in *Asclepias* as other than a normal condition.

The nucleus is too small for an accurate determination of the finer cytological phenomena at this or at later stages of division. After synapsis the reticulum opens out (Fig. 12), and stains deeply with the gentian, while the nucleolus stains less deeply than during synapsis. Fig. 16 shows what is

¹ e. g. in the generative cell of *Pinus*. Ferguson (1901).

probably an early stage in the segmentation of the spireme, but the details of this process could not be accurately followed.

Before the formation of the nuclear spindle, the nucleus elongates in the direction of the long axis of the mother-cell as described by Strasburger¹ for *Senecio vulgaris*.

The mode of disappearance of the nucleolus and of the nuclear membrane, and the origin of the nuclear spindle, were not observed, though careful search was made for them. The nuclear plate stage of the division is shown in Fig. 17. The long axis of the spindle is always parallel to the long axis of the cell, and does not vary as described by Juel (1900) for *Carex acuta*.

Fig. 17 *a* is a detail of this division. Here the U-shaped chromosomes at the equator of the spindle are shown to be twelve in number. Fig. 8 represents a tissue-cell in which twenty-four chromosomes are shown in the nucleus. After careful and extended counting, this has been determined to be the full complement for *Asclepias*, as Stevens also states. Fig. 3 *a* is a detail of the division cutting off the tapetal cell from the pollen-mother-cell. Here there are fifteen chromosomes shown, indicating that reduction in number does not take place in this division. It thus becomes evident that numerical reduction takes place in the division figured in Fig. 17.

Cell-division follows directly upon the division of the nucleus. The two daughter-nuclei form at the poles, and the cell-plate appears well defined. The daughter-nuclei become separated from the spindle-fibres very early, and the development of the cell-plate into a cell-wall takes place without any connexion with the daughter-nuclei (Fig. 18). This has been described by Stevens (1898), who showed its significance in the light of Haberlandt's² (1887) declaration that the influence of the daughter-nuclei propagated along the connecting fibres is necessary for the development of the cell-plate into the cell-wall.

Inspection of Figs. 18 and 20 shows delicate kinoplasmic

¹ 1879, p. 9.

² Quoted by Stevens.

radiations at the poles of the nuclear spindle. These radiations have been described for *Asclepias* by Raciborski (1897), and by Strasburger¹ as centrosomes. Raciborski says that if the sections are very thin and the lens strong enough, the supposed spherical centrosomes appear only as centres of radiating plasma-threads. My own slides agree with Raciborski's description.

THE SECOND DIVISION OF THE PRIMARY POLLINIUM-CELL.

The daughter-nuclei resulting from the first division of the primary pollinium-cell enter upon the resting stage (Fig. 19), with the chromatin distributed upon a linen reticulum. Gradually they take up a position at the centre of their respective daughter-cells, and in the meantime the spindle-fibres, which at first took a violet stain from the gentian-violet, lose this colour, and take on the yellowish brown colour of the cytoplasm. They gradually shorten and finally disappear (Fig. 20).

The nucleoli of the daughter-nuclei are very much smaller, relative to the size of the nucleus, than in the nucleus of the primary pollinium-cell. Stevens suggests from this fact that the substance of the nucleolus has gone to form the new structures in the cell. The writer found no direct evidence on this point.

The second division (Figs. 21, 22) is quite similar to the first. Synapsis was not observed and probably does not occur here. The divisions result in four cells, the structural units of the pollinium, arranged normally in a radial row. No exception to this was observed. The cytoplasm of the young pollinium-cell is much more fibrous in appearance than that of the mother-cell.

In no case were less than four pollinium-cells developed from each primary pollinium-cell, as Strasburger (1899) describes. In every instance each primary pollinium-cell divided and gave rise to four pollinium-cells arranged in a radial row (Fig. 23).

¹ 1900, p. 166.

The outer wall of the pollinium-cells consists of part of the wall of the mother-cell together with the cross walls formed by the division of the mother-cell. Neither the pollinium-cells nor the tetrads (as in Orchidaceae¹) ever become free, but form the pseudo-tissue known as the pollinium, a condition similar to that described by Rosanoff (1865) for Mimoseae. As they enlarge, these cells press upon each other and thus assume various shapes (Figs. 24-26, 32, 34), and the radial arrangement also becomes obliterated at last (Fig. 34). At places where the cytoplasm has shrunk slightly from the outer wall it is easy to see that a delicate inner membrane has been formed (Figs. 23-25).

At a very early stage, there appear in the cytoplasm clearly defined circular areas of rod-shaped bodies which stain very densely like chromosomes (Figs. 24, 25). In older pollinium-cells these bodies become more and more distinct (Fig. 26) and the oval spaces containing them seem to be delimited by a distinct membrane, though whether or not this is more than the surface film of the cytoplasm may be a question. Such a condition as that shown in Figs. 28-31 seems to indicate that there is a true membrane developed here about a vacuole in which are the rod-shaped bodies. These areas suggest the nuclear-like bodies described by Juel (1900) for *Syringa rothomagensis*, but of course it can be only a similarity in appearance, for Juel's interpretation is not at all probable for the condition found in *Asclepias*. These rod-shaped bodies gradually disappear as the pollinium-cells develop, and this suggests that their function is nutritive. They often descend into the pollen-tube, but have never been found to travel as far as the micropyle. It is possible that the disappearance of so large a percentage of the nucleolar substance of the primary pollinium-cell may have some connexion with the appearance of these bodies. The writer purposes to study them further as soon as fresh material again becomes available.

¹ Guignard (1882), p. 35.

THE FIRST DIVISION OF THE POLLINIUM-CELL.

The nucleus of the young pollinium-cell occupies an approximately central position in the cell. As the cell increases in size the nucleus enlarges considerably, and the nucleolus especially becomes relatively very large, and stains strongly with the gentian-violet. The chromatin granules are distributed evenly and apparently in a single row on the threads of the linin reticulum and stain deeply with the gentian.

As the time for division approaches, the nucleus migrates to the cell-wall of the pollinium-cell and becomes closely appressed to it, flattening out at the place of contact (Fig. 26). The nuclear membrane seems to disappear along this region, and the nucleus gives every indication of being firmly fixed to the cell-wall. Previous to the formation of the spindle, the nucleolus frequently fragments (Fig. 27), as in the case of the nucleolus of the mother-cell, but no wart-like bodies could be determined, and there was no suggestion as to the mode of fragmentation. There was no evidence that the nucleolus always fragments previous to this division.

The karyokinetic spindle is oriented at right angles to the cell-wall (Fig. 28), as described by Caldwell (1899) for *Lemna minor*, and Duggar (1900) for *Symplocarpus foetidus* and *Peltandra undulata*. It is probably of the 'multipolar diarch' type described by Strasburger (1900), though, on account of its minute size, this fact could not be determined with certainty. The spindle never becomes bipolar at either end as Caldwell and Duggar found, but the twelve chromosomes in the late anaphase stage are arranged at the ends of the spindle in rows parallel to the cell-plate (Fig. 29).

The formation of the cell-wall separating the two daughter-cells presents a very interesting figure. As the cell-plate lengthens, the ends of it bend toward the wall of the pollinium-cell in a manner similar to that described by Guignard (1882) for *Neottia Nidus avis*, by Duggar (1900) for *Symplocarpus*, and by Chamberlin (1899) for *Pinus Laricio*, forming an arc which cuts off a small lenticular cell. As the

cell-plate curves back, the spindle-fibres spread out and radiate in all directions from the cell-plate. The ends of the fibres, except those attached to the nucleus, are free in the cytoplasm of the larger cell. These fibres shorten and finally disappear as the cell-wall develops, and the smaller cell is cut off quite completely from the larger one, though it does not become entirely free at first, as it is held by the wall of the pollinium-cell at the two points where this is joined by the newly formed wall (Fig. 31 *a*). Eventually this connexion disappears, and the smaller cell, surrounded by its own cell-wall, lies, as it were, in the little pocket of its sister-cell. The development of this cell-wall from the cell-plate seems to take place here much as described by Mottier¹. By some unexplained process the smaller cell finally becomes completely surrounded by the larger one. It seems as if the larger cell grew around the smaller one, the cell-walls of the former breaking down at the point where they come in contact, for later, the smaller cell lies more deeply within the larger cell, and is surrounded by a membrane distinct from itself. No conclusive evidence, however, was found in support of this theory. The membrane is shown around the spindle in Fig. 32.

The nucleoli of both cells are relatively large and prominent objects, staining deeply with the gentian-violet (Fig. 31). No characteristic difference could be observed in the staining reaction of the two nuclei.

THE ORIGIN OF THE SPERM-CELLS.

The nucleus of the smaller cell divides in the pollinium-cell and, so far as observed, this division is accomplished before the germination of the pollinium-cells, as described by Schaffner (1896) for *Alisma Plantago*, Wiegand (1899) for *Potamogeton*, Merrell (1900) for *Silphium*, and others.

The nuclear spindle in this division is different from those observed in any of the other divisions. It is rather sharply

¹ Mottier (1897), p. 192.

pointed at both ends, and much longer and more acuminate at one pole than at the other (Fig. 32). The more tapering pole appears to be somewhat bent to one side, but this was doubtless caused by the shoving of the microtome knife, or by some other mechanical injury. The difference in the length and manner of tapering of the two ends does not seem to be due to the way the section was cut, as the poles seem well defined and pointed at both ends, and not truncate at one end as would be expected had the microtome knife cut off one pole. Here also the nucleus was too tiny to permit of the details of spindle-formation, &c., being clearly made out.

The nucleus of the larger cell at this period possesses a rather conspicuous, vacuolated nucleolus, with a well-defined membrane, and a prominent nuclear reticulum. It would seem as if this division takes place rather rapidly, for in over ten thousand pollinium-cells examined, at a time when this division would be expected, only the single instance figured was found.

Cell-division follows the division of the nucleus of the smaller cell, each daughter-nucleus organizing about itself a portion of protoplasm, forming two cells, which later prove to be the sperm-cells. These cells remain enclosed by the delicate wall which originally surrounded their mother-cell (Fig. 33). No instance was found where any of these pollinium-cells failed to develop, as Schleiden (1849) has described.

GERMINATION OF THE POLLINIUM-CELLS.

When the pollinia are deposited on the stigma, or placed in a suitable germinating medium, the outer wall bursts after a time, and the fertilization-tube protrudes (Figs. 34, 35). Brown (1833) states that this tube does not branch, but the writer found rare instances of the branching of the tube in the tissue of the style.

The nucleus of the larger cell enters the fertilization-tube in advance of the sperm-cells, and the latter remain close

together. Faint traces of the delicate wall that originally surrounded the smaller cell remain, even after the sperm-cells have advanced some way down the tube (Fig. 36), but finally the membrane completely disappears. Eventually the sperm-cells pass the tube-nucleus (Fig. 37), and are carried through the micropyle to the cavity of the ovule by the pollen-tube, which penetrates as far as the synergids. The fate of the tube-nucleus was not determined.

CONCLUSION.

There now remains the question first raised by Wille as to the true nature of the structures referred to above as pollinium-cells, and the homologies of the primary pollinium-cells. Juel's work on *Carex* has shown us that it is possible to have in the higher plants a structure physiologically a pollen-grain but morphologically something quite different. In fact, three alternatives may be stated as possible in the development of the sperm-cells in Angiosperms.

1. The normal development as described for most genera where the pollen-mother-cell, by two divisions at right angles to each other, gives rise to four pollen-grains, situated at the corners of a tetrahedron. Each grain ultimately develops two sperm-cells and a vegetative cell.

2. Division of the nucleus of the pollen-mother-cell into four nuclei, three of which abort, as in *Carex*. In this case only one pollen-grain develops from each mother-cell.

3. The development of the sperm-cells from the pollen-mother-cell without the so-called tetrad division of the latter, as suggested by Wille and others for *Asclepias*. This would be analogous to the development of the embryo-sac directly from the mother-cell in *Lilium*, but has not yet, so far as the writer knows, been demonstrated for any plant.

The second case is at once ruled out for *Asclepias*, since abortion of cells was not found at any stage in the development of the sperm-cells. Which of the two remaining cases, then, obtains here? Are the pollinium-cells pollen-mother-cells, as Wille and others have held, or true pollen-grains, as

is to be inferred from Stevens's statement? The answer to this question is found in the history of their development. In the case of the development of true pollen-grains, and of the spores of Bryophytes and Pteridophytes, numerical reduction of the chromosomes takes place in the mother-cell. In *Asclepias* this reduction occurs in the primary pollinium-cell, the two successive divisions giving rise to four cells, each of which eventually develops two sperm-cells and a larger cell which germinates into a tube through which the sperm-cells pass to the ovule. The course of development, so far as the number of nuclear divisions, the place of the numerical reduction of the chromosomes, and the mode of formation of the sperm-nuclei are concerned, agrees precisely with what takes place in other plants that form pollen-grains. We must conclude, therefore, that the primary pollinium-cells are pollen-mother-cells, and that the pollinium-cells are true pollen-grains.

The chief peculiarity in the division of the primary pollinium-cell is in the arrangement of the spindles of the successive mitoses. Two different orientations have hitherto been described in Angiosperms. First, spindles at right angles to each other; second, spindles parallel but not in the same line¹. In *Asclepias* we find a third variation, where the axes of the spindles are in the same line, resulting in the disposition of the four daughter-cells in a radial row. The only other instances of a similar arrangement are exceptions in the plants where they occur². This arrangement, however, would not affect the homology of the structures.

The close union of the pollinium-cells, each surrounded by a firm cellulose wall, and the fact that neither they nor the tetrads are ever set free, would be misleading in attempting to homologize them. Brongniart's sections of the pollen-mother-cells of *Cucurbita maxima* suggest in appearance the cross sections of the pollinium of *Asclepias*, yet Rosanoff has shown for Mimoseae a similar condition of pollen-grains.

¹ Coulter (1898) for *Ranunculus*; Fullmer (1899) for *Hemerocallis fulva*.

² Wimmel (1850) for *Fuchsia*; Wille (1886) for *Orchis mascula*.

SUMMARY.

1. The individual cells of the pollinium of *A. Cornuti* are true pollen-grains which never become free.

2. The divisions of the pollen-mother-cell are successive, and the spindles of the two divisions are oriented with their long axes radial and in the same line, so that each pollen-mother-cell gives rise to a row of four pollen-grains radially arranged.

3. The outer membrane of each pollen-grain is composed of the wall of the mother-cell (which does not dissolve), plus the cross walls formed by the two divisions of the mother-cell.

4. Each pollen-grain possesses an inner membrane which it develops about itself.

5. The generative cell divides, before the formation of the pollen-tube, into two sperm-cells, each of which travels down the pollen-tube, passing the vegetative nucleus on the way.

The above investigation was carried on in the Botanical Laboratory of Cornell University, and the writer wishes to express his best thanks and sincere appreciation to Professor G. F. Atkinson for exceptional opportunities offered by him, and for his constant encouragement and helpful suggestions.

ITHACA, N. Y.

Addendum :—Since the above paper was completed, Dr. Scott has called my attention to Corry's work on *Asclepias* (1883, 1884)¹. This writer agrees with Schacht in considering the archesporium as unicellular. From the archesporial cell are derived by successive divisions in different planes the pollen-mother-cells ('primitive mother-cells'). The pollinium is composed of the special mother-cells, which are formed by

¹ Corry, T. H., On the Mode of Development of the Pollinium in *Asclepias Cornuti*, Decaisne. Trans. Linn. Soc., London, 2nd Ser., Bot., vol. ii, p. 75, 1883.

— On the Structure and Development of the Gynostegium, and the Mode of Fertilization of *Asclepias Cornuti*, Decaisne (*A. syriaca*, L.). Trans. Linn. Soc., London, 2nd Ser., Bot., vol. ii, p. 175, 1884.

the division of the primitive mother-cells in three planes at right angles to each other.

Corry also observed two nuclei in the pollen-grain, but considered the smaller one the vegetative nucleus, and the larger one the 'active' nucleus. No mitotic figures are given by him, and he does not trace in detail the steps in the development of the pollen.

There have also appeared two other papers on *Asclepias* since my own paper was completed, one by Fry (1901)¹, and the other by Strasburger (1901)².

Fry considers the archesporium as multicellular, and states that the primary sporogenous cells become spore-mother-cells without further division. All the spore-mother-cells of a sporangium divide into four, but the exact number of chromosomes in any division was not determined. The generative and tube nuclei are figured, and the author considers that the generative nucleus moves to the wall of the pollen-grain before division, and is cut off by a cell-wall. The fact that this nucleus was formed at its lateral location as a result of the position assumed by the nucleus of the young microspore before division was not observed. Fry also mentions and figures the rod-shaped bodies in the pollen-grain that stain like chromatin, but tests with several reagents failed to reveal their nature.

Prof. Strasburger's paper is especially interesting from the fact that in 1889 he stated that the pollen-mother-cells never divide. In the first part of the paper is briefly discussed the literature concerning the homology between the pollen-mother-cell and the embryo-sac mother-cell, and the author refers to the fact that a shortening of the ontogeny, similar to that which takes place in the development of the embryo-sac of some plants, has never been observed in the development of pollen.

Strasburger then takes up the case of *Asclepias* and reviews

¹ Fry, T. C., Development of the Pollen in some Asclepiadaceae. Bot. Gaz., vol. xxxii, p. 325, 1901.

Strasburger, E., Eine Bemerkung zu der Pollenbildung bei *Asclepias*. Ber. d. deut. bot. Gesell., vol. vii, p. 450, 1901.

the literature, speculating on the probable development of the pollen-grain directly from the mother-cell. Then follow the results of his own researches. He considers the phenomenon of numerical reduction as the criterion for the pollen-mother-cell, and on this basis he considers the long cells which give rise to the individual cells of the pollinium as the mother-cells of the pollen. The full complement of chromosomes in *Asclepias* is held to be twenty, but the author states that he was unable to count them exactly. 'About ten' chromosomes were counted on the equatorial plate of the first division of the pollen-mother-cell.

The daughter-nuclei resulting from the first division of the pollen-mother-cell come to the full resting condition. The second division the author considers as 'homotypical,' but states that this cannot be proved on account of the smallness of the elements on the nuclear plate. Rod-shaped bodies are not figured, but the contents of many of the pollen-grains are described as especially coarse-grained, and eagerly devouring the stain. Possibly the rod-shaped bodies are here referred to.

The fact that the young pollen-grain divides into a larger vegetative and a smaller generative cell is mentioned and the completed division shown, but the karyokinesis is not figured.

Centrosomes were not found, and the author inclines very strongly to the opinion that they do not exist at all in the higher plants.

The above three papers all leave the identity of the pollen-grain and its ontogeny in *Asclepias* an open question, since the peculiarities of the division are so different from those observed heretofore in other types, and have led many to believe that a shortening of the process takes place in the members of this genus. It would remain an open question until it was proved that all the divisions concerned, up to and including the formation of the sperm-cells, are exactly the same in all essential points in *Asclepias* as those which occur in other Angiosperms. This identity is established for the first time by the developmental history as traced in the foregoing paper.

The full number of chromosomes was determined only after several hundred counts had been made, and Fig. 8, Plate VII, showing twenty-four elements on the division spindle seems almost conclusive evidence that the characteristic number for *Asclepias* is at least twenty-four.

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CORNELL UNIVERSITY,
December, 1901.

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EXPLANATION OF FIGURES IN PLATE VII.

Illustrating Mr. Gager's paper on the Pollinium of *Asclepias*.

All the figures are drawn with the aid of a camera lucida, at 140 mm. projection. Tube length 160 mm. Nos. 1 and 34 with Zeiss 8 ocular, and Bausch and Lomb 2-3 objective. Nos. 2, 3, 4, 5, 9, 17, 18, 20, 21, 22-25, 35, 37, with Zeiss 4 ocular, and 2 mm. hom. imm. Nos. 3a, 8, 17a, 21a, with Zeiss 18 ocular, and 2 mm. hom. imm. No. 6 with Zeiss 4 ocular, and Bausch and Lomb 2-3 objective. No. 7 with Zeiss 12 ocular, and Bausch and Lomb 2-3 objective. Nos. 10-16, 19, 26-32 with Zeiss 12 ocular, and 2 mm. hom. imm. Nos. 33, 36 with Zeiss 8 ocular, and 2 mm. hom. imm.

Fig. 1. Cross section of bud about 1 mm. in diameter, showing the five young anthers and the young primary pollinium-cells radially arranged in the developing microsporangia. $\times 85$.

Fig. 2. Cross section of portion of anther much younger than Fig. 1. Developing archesporium. $\times 450$.

Fig. 3. Cross section of portion of anther older than in Fig. 2. Division of archesporium to cut off tapetal cell. $\times 450$.

Fig. 3a. Detail of division figure in Fig. 3. Fifteen chromosomes on equator of spindle. $\times 1525$.

Fig. 4. Cross section of end of anther. Division complete, cutting off tapetal cell. $\times 450$.

Fig. 5. Cross section of end of anther. Young primary pollinium-cell with characteristic large nucleoli. Tapetal layer developing peripherally from the sister-cells of the primary pollinium-cells, axially from the parenchymatous cells. $\times 450$.

Fig. 6. Cross section of nearly mature anther. Primary pollinium-cells in first division. $\times 45$.

Fig. 7. Cross section of microsporangium. Tapetal layer well differentiated. Primary pollinium-cells in first division. $\times 120$.

Fig. 8. Tissue cell showing twenty-four chromosomes, the full complement for *Asclepias cornuti*. $\times 1525$.

Fig. 9. Young primary pollinium-cells, showing vacuoles in cytoplasm and in nucleolus, wart-like bodies, and first appearance of the small granules in the cytoplasm staining like chromatin. $\times 450$.

Fig. 10. Mature primary pollinium-cell. Fibrous character of the cytoplasm, and the small granules staining like chromatin. Nucleolus vacuolated and having a distinct membrane. Chromatin distributed on the threads of the nuclear reticulum. $\times 1225$.

Fig. 11. Synapsis preceding the first division of the primary pollinium-cell. $\times 1225$.

Fig. 12. Nucleus of the primary pollinium-cell soon after synapsis. Nucleolus vacuolated and having one wart-shaped body. $\times 1225$.

Figs. 13, 14. Nucleoli of primary pollinium-cells showing stages that suggest fragmentation and a possible connexion between fragmentation and the wart-like

bodies. The nucleoli have each one large vacuole. No. 14 had already given off one fragment. $\times 1225$.

Fig. 15. Nucleus of primary pollinium-cell showing six nucleoli. The larger one suggests a possible origin of the wart-like bodies. $\times 1225$.

Fig. 16. Nucleus of primary pollinium-cell showing early spireme. Two vacuoles in the nucleolus. $\times 1225$.

Fig. 17. First division of the primary pollinium-cells. $\times 450$.

Fig. 17a. Detail of first division of primary pollinium-cell, showing the reduced number (twelve) of chromosomes. $\times 1525$.

Fig. 18. Later stage in first division of the primary pollinium-cell. Cell-plate and kinoplasmic radiations from the daughter-nuclei. $\times 450$.

Fig. 19. Nucleus after completion of first division of primary pollinium-cell. $\times 1225$.

Fig. 20. Still later stage than Fig. 19 in first division of primary pollinium-cell. Cell-plate complete; daughter-nuclei moving to centre of daughter-cells. $\times 450$.

Fig. 21. Second division of primary pollinium-cell. $\times 450$.

Fig. 21a. Detail of second division of primary pollinium-cell, showing the reduced number of chromosomes, twelve at the lower pole, eleven at the upper. $\times 1525$.

Fig. 22. Second division of primary pollinium-cell, cell-plate stage. Kinoplasmic radiations from daughter-nuclei. $\times 450$.

Fig. 23. Second division of primary pollinium-cell complete, showing the four radially arranged tetrads. $\times 450$.

Fig. 24. Four tetrads from the same mother-cell, rounded, showing the delicate inner membrane and the early appearance of the rod-shaped bodies. $\times 450$.

Fig. 25. Young microspore showing further development of rod-shaped bodies. $\times 450$.

Fig. 26. Young pollinium-cell. The nucleus appears affixed to the cell-wall previous to the first division. The inner cell membrane and the rod-shaped bodies are prominent. $\times 1225$.

Fig. 27. Fragmentation of nucleolus before first division of the pollinium-cell. Cf. Fig. 15. $\times 1225$.

Figs. 28-31. The first division of the pollinium-cell. $\times 1225$.

Fig. 32. Division figure in the formation of the two sperm-cells. $\times 1225$.

Fig. 33. Division complete, forming the two sperm-cells. $\times 858$.

Fig. 34. Cross-section of pollinium. The pollinium-cells at the left have begun to germinate. $\times 85$.

Fig. 35. Germination of mature pollinium-cell. $\times 450$.

Fig. 36. Fertilization-tube germinated on the stigma. The tube-nucleus is in advance of the two sperm-cells. $\times 858$.

Fig. 37. Fertilization-tube about to enter the micropyle. The two sperm-cells have passed the tube-nucleus. Two synergids and oosphere in the embryo-sac. $\times 450$.

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32.

35.

37.

sp. c¹

sp. c²

vn.

b.

sp. c²

sp. c¹

vn.

vn.

gc.

vn.

vn.

syn.

en.

syn.

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