On the Life-history of *Salvinia natans*.¹

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With Plates XLI-XLIII and one Figure in the Text.

ALTHOUGH the germination of the micro- and macrospore and the embryo-formation of *Salvinia natans* had already been observed by several of the older investigators, such as Mettenius ('46), Hofmeister ('51, 57), and others, the first careful and complete account of the subject was given by Pringsheim in 1863. In his classical paper he followed pretty closely the development of the male and female gametophytes, and the formation of the embryo, besides describing very carefully the further development of the sporophyte. Pringsheim's paper may, therefore, be looked upon as the starting-point upon which all subsequent morphological studies on *Salvinia natans* are based.

In 1873 Juranyi published the results of his studies on the development of the micro- and macrosporangia and the formation of spores. These parts of the life-history of *Salvinia natans* were entirely neglected by Pringsheim, and Juranyi's paper may be considered as the first careful description of this subject. Three years later Arcangeli ('76) described the development of both sporangia and prothallia of *Salvinia*. He noticed the three layers, i. e. the pseudo-epispore, the exospore, and the endospore, in the spore-coat of both the macro- and microspore. The pseudo-epispore of the latter is considered by him to be common to all microspores. He also noticed that there are two antheridia in one male prothallium.

In 1879 Prantl's short paper appeared, dealing chiefly with the development of the female prothallium of *Salvinia*. He clearly demonstrated the existence of three spore-coats in the macrospore. In the same year Bauke ('79) described the further behaviour of the unfertilized prothallia of *Salvinia*. He confirmed and extended the statements of Pringsheim. A few years later Heinricher ('82) studied the development of the macro- and microspores, and corrected and extended the results of Juranyi.

¹ The substance of this paper has been already published in Japanese in the Botanical Magazine, Tokyo, vol. xxiv, 1910.

In 1887 Campbell, in a paper dealing with the development of plant-spermatozoids, described the development of the male gametophyte of *Salvinia*. He agrees with Pringsheim ('63), who stated that there is one antheridium containing eight spermatozoids in each male prothallium. Belajeff ('98) made a careful study of the development of the male prothallium of *Salvinia*. He mentions that there are two antheridia in a single male prothallium, differing from the statements of Pringsheim and Campbell. Campbell still maintains his older view in the second edition of 'Mosses and Ferns' (1905).

The material for the study of spore-formation was collected in October, and the gametogenesis, fertilization, and embryogeny were studied from the material raised from spores in the laboratory from January to March during the last two years. The material was fixed either with Flemming's weaker solution or with chromo-acetic acid mixture. It was embedded in paraffin with the melting-point 52° C. The studies were made almost entirely with microtome sections.

MICROSPORE FORMATION.

The microsporangium-initials are already formed on the surface of the very young sporocarp, as shown in the Text-figure. Each initial-cell undergoes a transverse division by which the stalk-cell is cut off from the apical cell (Pl. XLI, Figs. 1, 2). The stalk-cell then divides two or three

TEXT-FIGURE. Longitudinal section of a young sporocarp. *in*, indusium; *a*, air chambers; *m.i.*, microsporangium-initial.

times transversely. The apical cell segments again, cutting off the basal cell, the first primary wall-cell, below (Fig. 3, w_1). The apical cell then divides by a wall more or less perpendicular to that of the basal cell (Fig. 4, w_2). It is soon followed by a periclinal wall (Fig. 6, w_3), thus cutting off three primary wall-cells and the archesporial cell inside. According to Juranyi ('73), who made the first careful observations on the development of both the micro- and macrosporangium, one more wall-cell is cut off, thus forming four primary wall-cells

enclosing the archesporial cell instead of three as I have observed (compare Juranyi's Pl. I, Figs. 6, 7).

The first-formed cell of two upper primary wall-cells then divides by a wall parallel to the long axis of the sporangium (Fig. 7). Then another upper primary wall-cell soon divides by a similar wall. These cells divide further several times, thus contributing to the greater part of the

wall of the mature sporangium. The basal cell divides but few times, and forms the part of the wall attached to the stalk.

The archesporial cell divides by the periclinal walls, forming three primary tapetal cells and a central cell (Figs. 8, 9). The central cell now divides by a wall more or less parallel to the long axis of the sporangium. It is soon followed by the next division, which divides two cells simultaneously by walls perpendicular to the first (Figs. 10–12). The quadrant-cells, thus formed, are again divided into equal octants (Figs. 12, 13). Each of these eight cells divides once more, and the sixteen microspore-mother-cells are formed (Fig. 14). During the divisions of the central cell the tapetal cells also divide by radial walls, but never by periclinal walls, so there is always one layer of tapetal cells, not two as described by Juranyi (73). Juranyi adds, however, that the tapetal cells in the microsporangium are sometimes one-layered (Figs. 10–15).

The full-grown microspore-mother-cell has denser cytoplasmic contents and a much larger nucleus than the surrounding cells of the sporangium. Its nucleus contains a large nucleolus and a delicate linin-reticulum in which small chromatin-granules are embedded (Figs. 14, 18). The nucleus soon prepares for division and shows the characteristic synaptic contraction (Figs. 15, 17). The synapsis stage lasts comparatively long, and it is followed by the spireme which comes out from the contracted nuclear contents (Fig. 18). The spireme then becomes shorter and thicker, and at last it segments into chromosomes. After the chromosomes are formed, kinoplasmic fibres appear in the cytoplasm surrounding the nucleus. Then the nuclear membrane begins to disappear and the multipolar spindle enters the nuclear cavity and comes in contact with the chromosomes. The spindle, when it is completely formed, assumes a bipolar structure (Figs. 19–22).

The number of chromosomes is easily counted, as they are arranged at the equator of the spindle; the number is sixteen, and they are grouped in eight pairs (Fig. 21). Each half of the bivalent chromosomes now separates from the other and travels towards different poles of the spindle (Figs. 23, 24). When the daughter-chromosomes reach the pole they remain for a short time unchanged, and this stage is also favourable for counting their number (Figs. 24, 25).

As the spindle with a faint indication of a cell-plate at the equator disappears, two daughter-nuclei prepare for the second division. At the prophase of the division eight pairs of the chromosomes are distinctly visible (Fig. 27). The spindle is at first multipolar and later becomes bipolar. When the spindle is completely formed, eight bivalent chromosomes arrange themselves at the equator. The sister-chromosomes of the same pair then separate one from the other and travel towards the pole (Fig. 28). Each of the four granddaughter-nuclei thus formed receives

eight chromosomes. The walls are formed between the four nuclei, and thus four microspores are established (Pl. XLI, Figs. 29-32).

With the entering of the synapsis stage of the mother-cells, the tapetal cells commence to disintegrate, and soon the partition walls between individual cells disappear, the degenerating nuclei being embedded in the common cytoplasm (Fig. 15). As has already been described by Juranyi ('73) and Heinricher ('82), the fully-formed microspores separate from each other and lie free, side by side with the degenerating tapetal nuclei in the mass of the tapetal cytoplasm, which now completely fills up the inside of the sporangium (Figs. 33, 34).

The mature microspores later move towards the periphery of the sporangium, and the centre of the latter is occupied by a mass of vacuolated cytoplasm (Pringsheim ('63), Prantl ('79), Heinricher ('82)). No trace of disintegrating tapetal nuclei is found at this stage (Fig. 35). The mature microspore has a thin endospore and a thick exospore. It contains a rather large nucleus at or near the centre, and abundant starch-grains (Fig. 36).

MALE PROTHALLIUM.

The microspore begins to germinate inside the sporangium in the early spring. The material kept in the laboratory was found to commence germination at the end of January, and to continue it until about the middle of May.

The development and structure of the male prothallium agrees on the whole with the description of Belajeff ('98). Belajeff studied the prothallium *in toto* and used the plasmolysis method in differentiating the outline of individual cells. He does not seem to have observed any dividing figures in the developing prothallium, and his observations lack histological details.

The microspore first divides into two cells by a wall tangential to the surface of the sporangium (Figs. 37, 38). The inner or lower of the two cells then gives rise to the two unequal cells—the lower small root-cell and the upper large prothallium-cell (Fig. 39). Early investigators such as Pringsheim ('63), Arcangeli (76), and Prantl ('79), who made some errors in describing the structure of the male prothallium, failed to observe any root-cell. Campbell ('87), who has studied the spermatogenesis of *Salvinia* more recently, was also unable to find the root-cell. He states: 'Die Spore theilt sich durch eine Querwand und die untere der gebildeten Zellen bleibt ungetheilt, die vegetative Zelle des Prothalliums darstellend.' Belajeff ('98) was the first to demonstrate the presence of the root-cell, but he was not certain when it is formed.

After the first division or sometimes just before, the exospore splits along three radiating lines on the surface of the microspore. The section of the germinating microspore at this stage is shown in Fig. 37.

The uppermost cell of the young prothallium now divides into two cells by a more or less oblique cross-wall (Fig. 40), and the outer one of these is divided again by an oblique wall, forming an apical cell and an antheridium-mother-cell (Figs. 41-43). The inner cell is also divided transversely, giving rise to a sterile cell above and an antheridium-mother-cell below (Fig. 44). From each antheridium-mother-cell a small sterile cell or wall-cell is cut off by an oblique wall which extends from the side-wall to the lower transverse wall, and a larger cell, the central cell or sperm-grandmother-cell, is formed (Figs. 45-6). The antheridium is made up of a wall-cell and a central cell which later divides into four sperm-cells. A single spermatozoid is formed in each sperm-cell. Now the development of the male gametophyte, consisting of a root-cell, a prothallium-cell, an apical cell, and two antheridia which are separated by a sterile cell, is completed (Figs. 47-53).

In the dividing cell of the male prothallium, the number of chromosomes has always been found to be eight.

In his classical paper on *Salvinia natans* Pringsheim ('63) states that in a male prothallium a single antheridium with eight spermatozoids is formed. He gives a figure of the apical cell and two sperm-grandmother-cells, but he mentions neither the presence of the wall-cells nor the sterile cell. Arcangeli ('76) takes the two antheridial cells for two antheridia. Prantl ('79) seems to be of the same opinion; and he states: 'Ich glaube, die beiden vorderen Zellen ebenso gut als zwei Antheridia deuten zu können, deren jedes eine Gliederzelle des rudimentären Prothalliums einnimmt.' Campbell's ('87) description of the mature male prothallium is as follows: 'Das ganze Antheridium (wenn wir den ganzen oberen Theil des Prothalliums als ein einziges Antheridium ansehen) ist jetzt aus fünf oder sechs Zellen gebildet: zwei innern (Fig. 26, *m*), welche die Urmutterzellen der Spermatozoiden darstellen, einer Deckelzelle (*D*) und zwei oder drei anderen peripherischen Zellen (*P*).' His 'peripherische Zellen' correspond to the wall-cells, and are, according to him, two or three in number. However, when he found three wall-cells it is very likely, in my opinion, that he mistook the sterile cell for one of them (compare Campbell's Fig. 26). According to Belajeff ('98) each male prothallium contains two antheridia which are separated from each other by a sterile cell. In regard to this sterile cell, Campbell ('05) states as follows: 'This cell, however, did not occur in the specimen studied by me, where the two groups of sperm-cells were usually in immediate contact (Fig. 233, *E*).' In my material it was found that the sterile cell always separates the two groups of sperm-cells, and thus forms two antheridia in each prothallium instead of one, agreeing with the statement of Belajeff.

When the sperm-cell is completely formed, a blepharoplast appears in its cytoplasm, and soon elongates towards the nucleus (Fig. 52). In the

meantime the nuclear reticulum becomes more prominent, and the nucleus begins to elongate. The elongated blepharoplast attaches itself to the dorsal side of the nucleus, which now begins to coil. The elongating nucleus which forms the greater part of the spermatozoid-body makes a coil of about two turns (Pl. XLI, Figs. 50, 51, 53). The numerous cilia evidently originate from the blepharoplast attached to the dorsal side of the nucleus.

The spermatozoid, when it comes out from the sperm-cell, swims about by means of cilia attached to the anterior dorsal side of its body, making two spiral coils. It carries a vesicle in the hinder end of its body, which contains several small starch-grains; the vesicle may, sooner or later, be detached from the spermatozoid.

MACROSPORE FORMATION.

The macrosporangium-initials appear at the apex of the young sporocarp (Pl. XLII, Fig. 54). They are characterized by denser cytoplasm and larger nuclei. Each initial is cut off at the base by two oblique walls, which are more or less perpendicular to each other, and thus the young stalk is formed. The third wall, parallel to the first oblique wall, is then formed; the last-formed cell corresponds to the basal cell, the first primary wall-cell of the young microsporangium. The fourth wall, parallel to the second oblique wall of the stalk, is then formed, being soon followed by a periclinal wall, thus forming an archesporial cell and three primary wall-cells (Fig. 55).

Three primary cells of the tapetum are cut off by periclinal walls from the archesporial cell (Fig. 56). In the meantime the wall-cells are divided by repeated radial divisions. The primary tapetal cells also undergo repeated radial divisions, and one layer of the tapetum is formed (Figs. 57-60). According to Juranyi ('73) the tapetum usually consists of two layers, as the following statement shows: 'Die Mantelzellen theilen sich nämlich in tangentialer Richtung; dieser Theilungsweise sind aber dieselben zu dieser Zeit gewöhnlich nur einmal unterworfen, in Folge dessen der Mantel in der Regel nur zweischichtig wird (Taf. II, Fig. 22).' This statement is adopted by Campbell in his revised edition of 'Mosses and Ferns'. Heinricher ('82), however, mentions that the tapetum is usually of a single layer. I was not able to confirm the statement of Juranyi, and no case was met with in which the tapetum consisted of two layers of cells.

Simultaneously the central cell continues to divide (Figs. 57-59), and after the third division it reaches the spore-mother-cell stage; so there are eight spore-mother-cells in one macrosporangium. The spore-mother-cells are easily distinguished from the surrounding cells by denser granular cytoplasm and larger nuclei (Fig. 59). There are two different statements concerning the number of the macrospore-mother-cells. According to

Juranyi there are sixteen, while Heinricher gives them as eight. My observation agrees with that of Heinricher, i. e. the number is eight.

The resting nucleus of the macrospore-mother-cell contains a delicate reticulum, which may consist of a network of linin in which are embedded some small chromatin-granules and a large distinct nucleolus (Fig. 61). The reticulum then assumes a more or less thread-like structure, and gradually becomes located on one side of the nuclear cavity, thus entering the synapsis stage (Fig. 62). The synapsis stage lasts comparatively long, and is followed by the spireme stage. Although I was not able to find the double nature of the thread in the early stages of the spireme, it was more or less apparent in the later stages (Figs. 63, 64). The spireme then segments itself into eight chromosomes, each of which is bivalent in nature (Figs. 65, 66).

The formation of the spindle now begins. The spindle is at first multipolar in origin, and assumes later a bipolar structure (Figs. 67, 69). Chromosomes then arrange themselves at the equator of the spindle. The number of chromosomes can easily be counted at this stage when viewed from a pole of the spindle (Fig. 68). Each half of the eight bivalent chromosomes now travels towards the poles (Figs. 69, 70). A sign of longitudinal splitting is observed in the chromosomes before they reach the pole.

As the daughter-chromosomes reach the poles their number can often be counted, and is always found to be eight (Fig. 71), but soon they come closely together until the individual outlines are lost. The nuclear membrane then appears (Fig. 72).

The two daughter-nuclei then divide. The division is homotypic and results in the formation of four granddaughter-nuclei (Fig. 73-77).

Simultaneously with the synapsis stage of the mother-cell, the tapetal cells begin to disintegrate, and macrospores that have not yet separated from each other float in the cytoplasmic mass of the tapetal cells, as has already been noticed by Heinricher (Fig. 78).

Afterwards the original wall of the macrospore-mother-cells disappears and the macrospores are separated from each other (Fig. 79). A single macrospore is surrounded by a dense mass of the cytoplasm and becomes enlarged very rapidly, while the rest of the macrospores move near the periphery of the sporangium and finally disintegrate (Figs. 80-82). So it can be said that the functional macrospore is nourished by the degenerating sister-spores and tapetal cells.

The development of only one macrospore within each macrosporangium at the expense of the degenerating sister-spores and tapetal cells was noticed by Juranyi ('73). But his description concerning the disintegration of tapetal cells and abortive macrospores seems to be not quite correct, as has been pointed out by Heinricher ('82). My observations agree on the whole with the statement of Heinricher.

Although there is, as a rule, only one functional macrospore in each

macrosporangium, I have observed in a few preparations that two macrospores were becoming enlarged instead of one. It seems that even in those cases one of the two finally develops, at the expense of the other, into the mature macrospore.

The mature macrospore is surrounded by three membranes. The outermost membrane, the epispor, shows a vacuolate structure, and is not of equal thickness, being thickest at the top, where it is divided into three folds. The folds are formed by three ridges at the sides (Pl. XLII, Figs. 84, 85).

The structure and development of the spore-membrane have already been described by Mettenius ('46) and Pringsheim ('63). Some more details were later added by Juranyi ('73), Prantl ('79), and Heinricher ('82).

The formation of the epispor by the vacuolization and the transformation of the protoplasmic mass surrounding the macrospore have already been described by Heinricher ('82). Fig. 83 shows an earlier stage of the epispor formation, in which the least differentiated cytoplasmic mass with one of the developing nuclei is found at the periphery, while the inner portion is transformed into a mass of vacuoles. A little later stage is shown in Fig. 84. The cytoplasmic mass is almost entirely transformed into the vacuoles. According to Heinricher ('82) the smaller vacuoles are found near the periphery, while the larger ones are arranged near the inner side. I have found, however, that the larger vacuoles are found near the periphery rather than towards the centre. In the young epispor a thin layer of protoplasm with a number of nuclei is found inserted between each episporic fold and the underlying portion of the epispor; Fig. 85 shows a tangential section of the epispor through the episporic folds at the apex of a macrospore.

The next inner membrane, the exospore, is homogeneous in structure, and almost equal in thickness throughout. Although the origin of the exospore is not quite clear, I am inclined to think that it is formed from the protoplasmic mass surrounding the macrospore. The innermost endospore is very thin and directly lines the inner surface of the exospore.

FEMALE GAMETOPHYTE.

The macrospores germinate on the surface of the water after the wall of the sporocarp has decayed or even when it is only partly decayed. Macrospores used for the present study germinated during January and February in our laboratory. When germination begins, the cytoplasm at the upper part of the spore becomes very dense, and soon the nucleus is found to divide. One of the division-figures is shown in Fig. 86. A faint membrane is formed between the two resulting nuclei, thus dividing the whole macrospore into a smaller upper cell and a considerably larger lower cell.

The upper small cell forms, by further divisions, the prothallium tissue, while the nucleus of the lower large cell multiplies by free nuclear divisions, and the nuclei are finally distributed throughout the parietal layer of cytoplasm (Fig. 87). Before the prothallium pushes out from the spore cavity, a large cell appears on the upper surface of the prothallium, which is soon found to be the initial-cell of an archegonium (Fig. 88).

The archegonium-initial divides into two by a cross-wall (Pl. XLIII, Fig. 89), and the upper one is divided longitudinally twice (Figs. 90, 92, 94), by walls at right angles to each other, into four cells, every one of which is again divided twice transversely (Figs. 94-96). The uppermost four cells are cover-cells and the others are neck-cells.

Almost simultaneously with the first division of the upper cell, the lower one divides unequally by means of a curved wall into the smaller neck canal-cell and the larger central cell (Figs. 90-93). The central cell now divides by a curved cross-wall. The outer small cell is the ventral canal-cell and the inner large cell is the egg-cell (Figs. 94, 95).

Pringsheim gives no statement about the presence of the ventral canal-cell, but he seems to have observed it as his figures (Pringsheim '63, Pl. XXVI, Fig. 1) and the following statement shows: 'So sieht man, wie hier beiläufig bemerkt werden mag, den Inhalt der Canalzelle in zwei verschiedene Massen sich sondern (XXVI, 1), in einen grossen fädigschleimigen Klumpen, der die ganze Spitze ausfüllt, und einen kleinen tieferliegenden Klumpen, welcher der veränderte Zellkern der Centralzelle zu sein scheint.' The lower segment of the neck canal-cell in the above description may very likely represent the ventral canal-cell.

The nucleus of the neck canal-cell then divides into two, though there does not arise any membrane which would separate the nuclei. According to Campbell ('05), in *Azolla* 'the neck canal-cell may have its nucleus divide,' but mentions that 'this has not yet been observed in *Salvinia*'.

On both sides of this first-formed archegonium, there arise later two to four archegonia (Fig. 100). Pringsheim stated that on each female prothallium usually three archegonia arise, and he also mentions that he has sometimes seen the prothallium with a great many archegonia, which he attributes to the absence of fertilization. I have not been able to observe any prothallium with such numerous archegonia, as I have made no observation by preventing fertilization.

As already noticed by Pringsheim and later investigators of *Salvinia*, the vegetative cells of the prothallium contain many chlorophyll grains and continue to grow, until, after the appearance of the archegonia, they spread like wings on both sides of the prothallial tissue where the archegonia are located. The prothallium assumes, sooner or later, a dorsi-ventral structure, and the archegonia are found on its ventral side (Figs. 99, 100).

FERTILIZATION.

When the egg-cell becomes mature, the neck of the archegonium opens widely, and the slimy substance, resulting from the disintegration of the canal-cell and ventral canal-cell, comes out of the opening (Pl. XLIII, Fig. 98).

The cytoplasm of the egg-cell is often denser around the nucleus, showing a more or less fibrillar structure. The nucleus contains a large nucleolus and a faint, poorly developed reticulum (Fig. 96).

A great many spermatozoids enter the neck of the archegonium and reach the exposed surface of the egg-cell (Fig. 98). Normally only one of them penetrates the cytoplasm of the egg. Fig. 97 shows the egg-nucleus just after the entering of the sperm-nucleus. The latter appears as a short curved rod near the nucleolus of the former. The sperm-nucleus then seems to disorganize into a mass of granules of various sizes. Fig. 98 shows the sperm-nucleus about half-way disintegrated, closely attached to the nucleolus.

EMBRYO.

The first division of the fertilized egg was not found. The wall separating the two cells of the embryo is parallel to the axis of the archegonium (Figs. 101, 102). The next division in each of the two embryonal cells is transverse, and thus the quadrants are formed which represent respectively the stem, the cotyledon, the foot, and the root (Figs. 103, 105). The cells of the cotyledon and the stem quadrants have always denser cytoplasm than the other two quadrants.

The cells of the quadrant both of cotyledon and stem divide by walls perpendicular to both of the first two walls (Fig. 104). In the stem-quadrant one of the two cells now formed becomes an initial of the apical cell, and it divides by a more or less oblique transverse wall, thus cutting off an apical cell above. The other cell of the stem-quadrant also divides like its sister-cell, and later develops into the first leaf of the young stem.

The early divisions in the cells of the cotyledonary quadrant do not differ much from those of the stem-quadrant mentioned above. But later they develop into a quite different structure, and thus, growing rapidly towards both sides and below, a triangular cotyledon is formed (Figs. 106-111).

In the root- and foot-quadrants the cell-divisions are not so regular as those of the stem or cotyledon. The development of the root ceases at an early stage, and later its tissue cannot be distinguished from that of the foot. The foot, on the other hand, develops to a massive structure. The lowest cells of the foot are filled with rather dense cytoplasm.

Pringsheim ('63) mentions that the triangular shape of the cotyledon is due to the presence of the three apical cells at its three corners, but I cannot confirm his statement. The cell at each corner of the triangle is not

an apical cell, being older than the surrounding cells, and ceasing to divide in the early stage of development (Figs. 109, 110). I found that two apical cells lying rather close to each other are situated at the upper sinus of the cotyledon, i.e. at the middle of the base of the inverted triangle.

The further development of the embryo is described in detail by Pringsheim, and I have nothing to add to his statement, as I can only confirm it.

SUMMARY.

1. The primary tapetum-cells of both sporangia are formed by division of the archesporial cell, and they again divide into one layer of many cells.

2. The spore-mother-cells are eight in the macrosporangium and sixteen in the microsporangium.

3. The number of chromosomes of the spore-mother-cell is sixteen and the reduced number in the spore is eight.

4. During the reduction-division the tapetum-cells begin to degenerate and nourish the spores.

5. Sixty-four mature spores are formed in a microsporangium, but in a macrosporangium only one spore becomes mature.

6. The male prothallium consists of a large prothallium-cell, a small root-cell, two sterile cells and two antheridia, which consist of a wall-cell and a central cell. The central cell divides twice, and in each of the four cells a spermatozoid is formed.

7. The statement of Campbell, who denies the presence of a sterile cell between the two antheridia and the root-cell, was not confirmed, in agreement with the investigation of Belajeff. Belajeff was not clear about the time of the appearance of the root-cell. It has been determined by the present study that the root-cell is formed as a result of the second division of the germinating microspore.

8. The spermatozoid is a spirally coiled body having numerous cilia at the anterior end, and a large vesicle at the posterior end.

9. A blepharoplast, which first appears in the cytoplasm and later elongates towards the nucleus, was observed in each sperm-cell.

10. Three to five archegonia are usually formed in each female prothallium, and each archegonium consists of one egg-cell, a ventral canal-cell, a neck canal-cell with two nuclei, and the neck-cells.

11. Only one spermatozoid enters into the egg-cell. After the penetration of the spermatozoid into the egg-nucleus, there appears in the cavity of the latter a nucleolus-like body, derived very likely from the spermatozoid and somewhat smaller than the nucleolus.

12. The four quadrants are clearly distinguishable in the young embryo. The development of the root-quadrant ceases at an early stage, and later its tissue cannot be distinguished from that of the foot.

I am indebted to Dr. K. Miyake for his kind suggestions and advice during the progress of this work.

Note.—After this had been written, Professor Arnoldi's paper, 'Beiträge zur Morphologie der Keimung von *Salvinia natans*,' appeared in *Flora* (Bd. c, pp. 122–139, 1909). His results agree with mine in essential points, except in one important matter, i.e. the number of chromosomes. He gives four as the chromosome number of the gametophytes and eight for the sporophyte. I have found, however, that the number is twice as many as that given by Arnoldi, being eight and sixteen respectively.

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EXPLANATION OF PLATES XLI–XLIII.

Illustrating Miss Yasui's paper on *Salvinia natans*.

All figures are drawn with the aid of a camera lucida, from microtome sections.

PLATE XLI.

Fig. 1. Longitudinal section of the initial cell of a macrosporangium. $\times 600$.

Fig. 2. Longitudinal section of the initial cell of a microsporangium which is cutting off a stalk-cell. $\times 600$.

Fig. 3. The first wall-cell (w_1) is cut off from the apical cell. $\times 600$.

Fig. 4. The second wall-cell (w_2) is cut off from the apical cell. $\times 600$.

- Fig. 5. Cross-section of the same stage as above. $\times 600$.
- Fig. 6. The third wall-cell (w_3) is just formed; the archesporium is now surrounded by three wall-cells. $\times 600$.
- Fig. 7. *a*, cross-section of a young microsporangium in which the second wall-cell is divided into two; *b*, the upper surface view of the same. $\times 600$.
- Fig. 8. The first tapetum-initial (t_1) is segmented from the archesporium. $\times 600$.
- Fig. 9. The third tapetum-initial is cut off from the archesporium; t_3 , the nucleus of the third tapetum-initial. $\times 600$.
- Fig. 10. The second division of the central cell. $\times 600$.
- Fig. 11. The same as above. $\times 600$.
- Fig. 12. A later stage; the four cells are formed. $\times 600$.
- Fig. 13. A still later stage; the eight cells are formed. $\times 600$.
- Fig. 14. Cross-section of a microsporangium in which sixteen spore-mother-cells are formed $\times 600$.
- Fig. 15. Cross-section of a microsporangium in which the microspore-mother-cells are at the synapsis stage and the tapetum-cells are degenerating. $\times 600$.
- Fig. 16. A microspore-mother-cell in the resting stage. $\times 1400$.
- Fig. 17. The microspore-mother-cell in synapsis. $\times 1400$.
- Fig. 18. A microspore-mother-cell in spireme stage. $\times 1400$.
- Fig. 19. Multipolar spindle stage. $\times 1400$.
- Fig. 20. The same as above. $\times 1400$.
- Fig. 21. Cross-section of the equatorial plate. $\times 1400$.
- Fig. 22. Longitudinal section of a heterotypic spindle. $\times 1400$.
- Fig. 23. A later stage of the same. $\times 1400$.
- Fig. 24. The same in telophase. $\times 1400$.
- Fig. 25. A later stage; the reconstruction of the daughter-nuclei has begun. $\times 1400$.
- Fig. 26. Two daughter-nuclei are just formed. $\times 1400$.
- Fig. 27. Prophase of the second division. $\times 1400$.
- Fig. 28. A later stage of the second division. $\times 1400$.
- Fig. 29. The telophase of the second division. $\times 1400$.
- Fig. 30. The wall formation between four granddaughter-nuclei. $\times 1400$.
- Fig. 31. Young microspore just formed. $\times 1400$.
- Fig. 32. Young free microspore. $\times 1400$.
- Fig. 33. A microsporangium containing microspore tetrads. $\times 600$.
- Fig. 34. A later stage; individual spores are separated from each other. $\times 600$.
- Fig. 35. Longitudinal section of mature microsporangium, showing microspores at the periphery. $\times 600$.
- Fig. 36. A mature microspore. $\times 1400$.
- Fig. 37. A germinating microspore; the nucleus is in division. $\times 1400$.
- Fig. 38. The first division is completed. $\times 1400$.
- Fig. 39. The lower of the two cells is dividing to form the root-cell below. $\times 1400$.
- Fig. 40. A little later stage; the upper of the two cells has already divided into two. $\times 1400$.
- Fig. 41. The division of the apical cell. $\times 1400$.
- Fig. 42. A later stage of the same. $\times 1400$.
- Fig. 43. A still later stage. *a.c.*, apical cell; *a.m.c.*, antheridium-mother-cell. $\times 1400$.
- Fig. 44. The lower antheridium-mother-cell, being separated from the upper antheridium-mother-cell by a sterile cell, is differentiated. *s.c.*, sterile cell. $\times 1400$.
- Fig. 45. The wall-cell (*w.c.*) of the upper antheridium is formed. $\times 1400$.
- Fig. 46. The wall-cell of the lower antheridium is formed; the root-cell (*r.c.*) is shown. $\times 1400$.
- Fig. 47. The nucleus of the central cell of the upper antheridium is just divided, while that of the lower antheridium is in the telophase of the division. $\times 1400$.
- Fig. 48. A slightly later stage of the same. $\times 1400$.
- Fig. 49. The last division in the central cell of the lower antheridium. $\times 1400$.
- Fig. 50. A nearly mature male prothallium; spermatozoids are developing in the sperm-cells of both antheridia. $\times 1400$.
- Fig. 51. About the same stage as the preceding; the root-cell is shown. $\times 1400$.

Fig. 52. Cross-section of an antheridium, showing an elongating blepharoplast in each sperm-cell. $\times 1400$.

Fig. 53. A mature male prothallium; the root-cell is not shown. $\times 1400$.

PLATE XLII.

Fig. 54. The initial-cell of a macrosporangium. $\times 600$.

Fig. 55. Three young macrosporangia in various stages of development. $\times 600$.

Fig. 56. A young macrosporangium in which the tapetum-initials are cut off. $\times 600$.

Fig. 57. A little later stage in which the central cell is divided once. $\times 600$.

Fig. 58. The second division of the central cell. $\times 600$.

Fig. 59. Eight macrospore-mother-cells are formed. $\times 600$.

Fig. 60. Cross-section of a macrosporangium, in which the macrospore-mother-cells are in the synapsis stage. $\times 600$.

Fig. 61. A macrospore-mother-cell whose nucleus is in the resting stage. $\times 1400$.

Fig. 62. Macrospore-mother-cell whose nucleus is in the synapsis stage. $\times 1400$.

Fig. 63. The same in early spireme stage. $\times 1400$.

Fig. 64. A later stage of the same. $\times 1400$.

Fig. 65. Three macrospore-mother-cells in which the chromosomes are just formed. $\times 1400$.

Fig. 66. A slightly later stage; the diakinesis. $\times 1400$.

Fig. 67. The formation of the multipolar spindle. $\times 1400$.

Fig. 68. Cross-section of an equatorial plate, showing eight bivalent chromosomes. $\times 1400$.

Fig. 69. Spindle of the first division. $\times 1400$.

Fig. 70. The same as above. $\times 1400$.

Fig. 71. Telophase of the first division. $\times 1400$.

Fig. 72. The two daughter-nuclei of the first division. $\times 1400$.

Fig. 73. The prophase of the second division. $\times 1400$.

Fig. 74. The telophase of the second division. $\times 1400$.

Fig. 75. The granddaughter-nuclei are formed. $\times 1400$.

Fig. 76. Macrospore-tetrad. $\times 1400$.

Fig. 77. Young macrospores. $\times 1400$.

Fig. 78. A macrosporangium containing macrospore-tetrads. $\times 450$.

Fig. 79. A later stage with free macrospores. $\times 450$.

Fig. 80. The functional macrospore (*m.*) is located at the centre, and all the other potential macrospores are moving towards the periphery of the macrosporangium. $\times 450$.

Fig. 81. A later stage; the functional macrospore is surrounded by the cytoplasm and nuclei of disorganized tapetum-cells. $\times 260$.

Fig. 82. A still later stage. $\times 260$.

Fig. 83. Radial section of the spore-coat of a young macrospore. $\times 600$.

Fig. 84. Radial section of the spore-coat of an older macrospore; *n*, nucleus of the tapetal cell; *ep*, epispore; *ex*, exospore. $\times 260$.

Fig. 85. The tangential section of the same. $\times 260$.

Fig. 86. The first division of a germinating macrospore. $\times 800$.

Fig. 87. Upper part of a young female prothallium. $\times 800$.

Fig. 88. The initial-cell of an archegonium. $\times 800$.

PLATE XLIII.

Fig. 89. A young archegonium; a primary neck-cell is just formed. $\times 800$.

Figs. 90–92. The division of the central cell. $\times 800$.

Fig. 93. The division is completed and the neck canal-cell is formed above. $\times 800$.

Fig. 94. The ventral canal-cell is formed by the second division of the central cell. $\times 800$.

Fig. 95. The division of the nucleus of the neck canal-cell. $\times 800$.

Fig. 96. A mature archegonium. $\times 800$.

Fig. 97. An egg-cell just after the entrance of a spermatozoid. $\times 800$.

Fig. 98. An archegonium, showing numerous spermatozoids entering it; the egg is already fertilized. $\times 800$.

Fig. 99. The upper part of a germinating macrospore, showing young prothallium with an archegonium. $\times 100$.

Fig. 100. Longitudinal section of a prothallium, with five archegonia. $\times 100$.

Fig. 101. A fertilized egg-cell, preparing for the first division. $\times 600$.

Fig. 102. Two-cell stage of the embryo. $\times 600$.

Fig. 103. One of the two cells is dividing. $\times 600$.

Fig. 104. The stem and cotyledonary quadrant-cells are dividing. $\times 600$.

Fig. 105. The four-cell stage. $\times 600$.

Fig. 106. The upper two cells are divided by cross-walls. $\times 600$.

Fig. 107. An apical cell is formed in each stem and cotyledon quadrant. $\times 600$.

Fig. 108. A later stage. $\times 600$.

Fig. 109. A still later stage. $\times 600$.

Fig. 110. Longitudinal section of a young plant. $\times 360$.

Fig. 111. Longitudinal section of the cotyledon. $\times 360$.

Fig. 112. Two embryos in the same prothallium. $\times 600$.

Fig. 113. A cell of a young sporophyte. $\times 1400$.

Figs. 114-119. Various stages of the division of the cells of a young sporophyte. $\times 1400$.

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