

II. *On the Development of the Ovule in Orchis Morio, Linn.*

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IN the spring and summer of last year I made many observations on the young ovules of various plants, with the view of testing the various doctrines on this subject, which had acquired new interest from the recent researches of Amici, Mohl and others. Only one series of my investigations attained anything like completeness; but in *Orchis Morio* I believe that I have seen and can confirm all that the above-mentioned observers have described; and I now present my results to the Linnean Society, partly because I believe that in the present state of the question all evidence derived from careful observation is of some value, and partly because I have succeeded in obtaining a more complete series of figures illustrating the successive conditions of the ovule than has yet been published; Mohl, who gives the most complete account of the development in *Orchis Morio*, having given no drawings. The following account is drawn up from my notes made during the observations, principally in the month of May 1848.

May 3rd. In the ovaries of flowers which had just opened, and were without signs of pollen upon the stigmatic surface, the ovules, about  $\frac{1}{200}$ th of an inch long, were just curving over toward the anatropous position; in some the axis of the nucleus formed nearly a right angle with the funiculus (TAB. II. figs. 4 & 5). The nucleus projected beyond the cells, forming the single coat of the ovule, and consisted of a large central cell (the embryo-sac), enclosed by a layer of very delicate cells of small size, constituting a proper coat of the nucleus.

May 9th. The ovules of fully expanded flowers were not much altered, except in the much clearer definition of the walls of the cells. The embryo-sac was filled with a clear, colourless fluid, in which floated minute black atoms, scarcely large enough to deserve the name of granules. In some flowers the stigmas were smeared with pollen, but often from the anthers of other flowers, their own being still closed. These pollen masses sent down numerous tubes, which differed much from any of the cells of the tissue in which they were engaged. The pollen-tubes were always about  $\frac{1}{400}$ th of an inch in diameter, at most one-fourth of the size of the smallest of the surrounding cells, which were also short and often irregular in form, while the pollen-tubes always appeared as long, slender filaments.

May 13th. The flowers withered and the stigmas covered with pollen. A dense bundle of tubes lay in the midst of the lax tissue of the canal leading to the cavity of the ovary. The ovules were considerably advanced, some being quite anatropous (fig. 6), others three-fourths reversed; those quite anatropous were about  $\frac{1}{100}$ th of an inch in length. The two coats of the ovule (tegmen and testa) were now distinctly evident; the length of the testa

varied; sometimes it half enveloped the tegmen, in some ovules it had grown up further over it. The inner coat, the tegmen, had not grown over the nucleus in all the ovules, but in most it projected beyond. The nucleus was still covered by its own cellular coat, and still contained only the clear, colourless fluid with black points.

May 16th. The ovaries more advanced; the pistillary cords extended nearly to the base of the ovary, lying in the grooves formed between the projecting placentas and the walls of the ovary, apparently free, and composed of delicate tubes presenting all the characters of pollen-tubes, and apparently continuous with these, as derived from the pollen on the stigma. The ovules (fig. 8) exhibited considerable alteration. Most of them were enlarged, and the outer coat had developed much in the chalazal region; its cells were larger and more clearly defined. The inner coat, which appeared to be tolerably independent of the outer at the sides, as air passed freely between them, had grown up far beyond the nucleus, and its cell-walls had acquired more consistence. The nucleus was much changed; the embryo-sac had lost its proper cellular coat, which had disappeared either by solution or by pressure, probably the former, as a free space existed sometimes between the inner coat and the nucleus; and in some cases the solution appeared imperfect, extending only to the cross walls of the cells, so that the embryo-sac was contained in an outer sac consisting merely of the outer walls of the cells of its coat. The embryo-sac now had the aspect of a large ovoid sac attached by a cellular pedicle to the chalazal region, and contained opalescent mucilaginous matter (protoplasm), in most cases accumulated at the ends, chiefly at that next to the micropyle. There was no sign of a nucleus or nascent cell yet.

May 20th. The embryo-sacs exhibited the collections of protoplasm at the two ends. At the micropyle end new phænomena presented themselves: either one, two, or (and usually \*) three minute vesicles (figs. 11–14) had been formed from the protoplasm, and always seemed to me to originate as cavities excavated in the mucilage, not as if formed by the formation of membrane on the outer surface of a nucleus (cytoblast) or globule of mucilage. These vesicles soon appeared as distinct cells, with exceedingly delicate walls, lying at the micropyle end of the embryo-sac, and undoubtedly existed there before the pollen-tubes entered the foramina of the ovules.

In some of the ovules examined this day the pollen-tubes had entered the ovules, and I traced them down through the wide mouth of the outer coat and the narrow canal of the inner, as far as the apex of the embryo-sac. They never entered this, but generally appeared to be diverted a little to one side, and to lie in contact with its outer surface †, just over the place where the minute vesicles lie within.

May 31st. I examined a number of ovules in various stages, repeating the observations on the earlier conditions with similar results. I traced the pollen-tube down to the embryo-sac in several specimens (fig. 15): in one case it appeared flattened against the membrane of the embryo-sac (fig. 17); in other cases (figs. 15, 16, 19, 20) I traced it a little way

\* It is probable that there are always three; but as they vary in size and lie close together, one or even two of them may be hidden in certain cases.

† The end of the pollen-tube exhibits dark contents when in contact with the embryo-sac.

down the side of the summit of the embryo-sacs, which always contained the vesicles within. In some embryo-sacs (figs. 20–26) one of the vesicles had begun to develop further, dividing into two cells by a horizontal septum, the upper dividing again and growing out in a conical form through the endostome, to produce the confervoid filament which was described by Mr. Brown, and which Schleiden has certainly mistaken for a development of the pollen-tube.

June 3rd. Traced the pollen-tubes to the embryo-sac, and saw them lying on the outside, and again satisfied myself that the vesicle within the embryo-sac (the germinal vesicle) is the first cell of the embryonic body. It generally exhibits a slight collection of protoplasm at its base, and soon after the pollen-tube reaches the surface of the embryo-sac divides into two cells, the upper dividing again and growing out into an articulated filament, the cells of which are formed by the production of septa in the same way as in *Confervas*, hairs of *Phanerogamia*, &c., the mucilaginous layer (or primordial utricle of Mohl) being rendered very evident by the application of iodine (fig. 29). The lower part of the embryonic body enlarges while the filament is growing out, and soon perfectly fills the embryo-sac. It appears to me that the process of cell-formation in this lower part, by which the embryo is produced, varies in different cases; generally the lowest cell enlarges very much and becomes filled with dark mucilaginous matter, and then this is soon divided into a number of cells by the formation of septa. Nuclei were visible in all the cells very soon after their origin, but I could not form an opinion as to their relation to the cell-formation, or determine how or at what period they were really produced. In the earliest condition they resembled clear vesicles, not granular bodies such as Schleiden describes.

In some cases two confervoid filaments are produced, two of the germinal vesicles undergoing development. I met with this several times, but omitted to draw them, in the hope of subsequently finding a more favourable specimen, which I was not fortunate enough to do.

The obvious conclusions from the foregoing observations appear to be, that the embryo is really produced by the ovule itself; that a germinal vesicle exists within the embryo-sac before the pollen exerts its influence; that the pollen-tube penetrates the coats of the ovule to reach the embryo-sac; and that the passage of the pollinic fluid through the intervening membranes impregnates the germinal vesicle and determines its development into an embryo.

Since the investigations were made with every precaution, and their results are in perfect accordance with those of Amici, Mohl, Müller and others, I think that I am justified in believing them to be a sufficient refutation of Schleiden's views, so far as the plant in question is concerned; but as to their positive value, as to the evidence they afford of the actual nature of the process of impregnation, I still regard them as insufficient. I am not convinced that the whole of the pistillary cords are composed of filaments directly produced by the pollen-granules. It is not yet shown whether there is any relation between the application of the pollen on the stigma and the development of the germinal vesicles; it is only clear that these last exist before the pollen-tubes enter the ovules.

Lastly, although the production of the confervoid filaments appears to be a normal process, it is still a question open to doubt when only observed in ovaries containing such an abundance of ovules as *Orchis Morio*.

The facts I have detailed above are, however, agreeable with what I have observed in certain other plants, in some as yet imperfect investigations; I hope to be able to complete them, and to repeat the earlier examinations with especial reference to the doubtful points, in the course of the ensuing summer.

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## EXPLANATION OF THE PLATE.

### TAB. II.

(The Figures are all magnified about 200 times.)

#### *Orchis pyramidalis*.

Fig. 1. A young ovule.

Fig. 2. The same, somewhat more advanced. The ovule presents a single coat, enclosing the nucleus, which consists of a layer of cells (the coat of the nucleus), surrounding a large central cell (the embryo-sac).

Fig. 3. An end view of the summit of the last.

#### *Orchis Morio*.

Fig. 4. A young, almost erect, ovule with a single coat, from which the nucleus projects.

Fig. 5. A more advanced ovule, curving round and exhibiting the nucleus and embryo-sac more distinctly.

Fig. 6. More advanced stage, ovule almost anatropous; both coats are now distinguishable, the inner projecting out from the outer, and the nucleus beyond the inner.

Fig. 7. The inner coat has grown over the nucleus, which still retains its proper cellular coat (7 a).

Fig. 8. The outer coat has grown up further; the nucleus has lost its coat, and is now a simple sac filled with a clear fluid in which float black granules (8 a).

Fig. 9. The outer coat almost completely covers the inner, which, with the nucleus, is indicated by dotted lines. The endostome is now very narrow; the nucleus contains mucilaginous matter (protoplasm, 9 a).

Fig. 10 to 14. Embryo-sacs from ovules a little more advanced than in fig. 9, exhibiting the vesicles at the micropyle end.

Fig. 15. An ovule with the pollen-tube penetrating. The exostome is a wide mouth, the endostome very narrow. The blind extremity of the pollen-tube lies upon the outside of the embryo-sac, within which is seen one large germinal vesicle.

Fig. 16 to 22. Embryo-sacs with pollen-tubes in contact, and with germinal vesicles within. In

Fig. 20 & 21 the germinal vesicle has divided into two cells by the formation of a transverse septum.

Fig. 23 to 29. Different stages of development of the confervoid filament from the pro-embryo. In fig. 25 the pollen-tube lies beside it. In fig. 29 the upper cells of the filament exhibit the contracted mucilaginous layers (primordial utricles) detached from the cell-walls. The lower part, which produces the embryo, is filled with opaque mucilage, which appears to divide into separate cells in various ways.

