Double Fertilization in a Dicotyledon— *Caltha palustris* ¹.

BY

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With Plate XXX.
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THIS striking and hitherto unknown phenomenon of double fertilization was, I think, first discussed in England after the publication of Professor Léon Guignard's paper on *Lilium Martagon* and *L. Pyrenaicum* in the spring of 1899 ². In August of the preceding year, however, Professor S. Nawaschin had given an account of his new researches on *L. Martagon* and *Fritillaria tenella* before the Russian Scientific Congress held at Kief ³.

It will be remembered that upon the expulsion of the contents of the pollen-tube into the embryo-sac of any typical Angiosperm, one of the two generative nuclei fuses with the nucleus of the oosphere, but the other has never been satisfactorily accounted for, and it was presumed that it became disorganized as the synergids do. Nawaschin, however, found in the plants mentioned that this second generative nucleus

¹ E. N. Thomas, 'On the presence of vermiform nuclei in a Dicotyledon.' Note, *Annals of Bot.*, June, 1900.

² M. L. Guignard, 'Sur les anthérozöides et la double copulation sexuelle chez les végétaux angiospermes.' *Comptes rendus*, t. cxxviii, Avril, 1899.

³ S. Nawaschin, 'Resultate einer Revision der Befruchtungsvorgänge bei *Lilium Martagon* und *Fritillaria tenella*.' *Bull. de l'Acad. des Sc. de St. Pétersbourg*, 1898, novembre.

travels to the middle of the sac, and there coalesces with the upper polar nucleus, and then both fuse with the lower polar nucleus. Guignard agrees with this, and furthermore states that the polar nuclei may have approached¹ one another before the generative nucleus joins them, and gives many figures showing the triple fusion. He gives instances also in which, when the polar nuclei are still separate, the generative nucleus joins the lower one. However this may be, the definitive nucleus which later gives rise to the endosperm is the product of the fusion of three nuclei of very different origin. The upper polar nucleus is sister-nucleus to that of the oosphere, and were it not for the presence of the lower polar nucleus, which is vegetative in character, the fusion of the second generative nucleus with the upper polar nucleus would be a true fertilization precisely equivalent to the fertilization of the oosphere by the male nucleus.

In addition to this most important discovery, both observers found that the generative nuclei had a very remarkable shape. They are long and narrow, and twisted in every conceivable manner, so that the term vermiform has been applied to them, and their appearance suggested to Guignard their strict analogy to antherozoids. The contortions are particularly noticeable in the second generative nucleus on its journey to the polar nuclei, and no doubt indicate the wriggling movement by which it travels through the sac.

When these accounts were published, Miss Sargent found that she had preparations of *Lilium Martagon* made years before which showed the vermiform nuclei and double fertilization extremely well², and as she still possessed the material which furnished these slides, she proposed that I should cut some of it to see how readily this stage could be obtained. As it was not convenient in some ways to work at Reigate, Professor Farmer very kindly consented to my working at the Royal College of Science, South Kensington, and throughout has given me the greatest assistance. I went there in May of

¹ Comptes rendus, p. 4 of separate copy.

² Sargent, Proc. Roy. Soc., May, 1899.

last year, quite prepared to extend my researches to other plants if *L. Martagon* proved fruitful speedily. The material must have been extremely rich in this stage, and very well fixed, for both hand sections and microtome series yielded very good instances at once.

As the matter seemed fairly well established for typical Monocotyledons, it became desirable to see if the second fertilization took place in Dicotyledons also. At Professor Farmer's suggestion I sectioned *Caltha palustris* among other plants, and this seemed to be a favourable object for study, although too old. I therefore went out in search of fresh material, and obtained it from Hackbridge, where it grew very abundantly. The ovaries were pickled on one or two bright mornings (11.30 and 12.30) at the end of May, some in absolute alcohol and 25% acetic acid, and some in strong Flemming's fixative. Those chosen were mostly from fresh flowers, but of different ages, as far as one could judge from their appearance. I know now, since carefully marking flowers of *Caltha* this spring, that they remain open for at least seven days, and this material must therefore have included ovaries of very different ages. Microtome sections were cut, for the most part, from the Flemming material, and hand sections from that fixed in absolute alc. and 25% acetic acid, but curiously enough the most interesting preparations were obtained from microtome sections of ovaries from the latter material, so that these are not so well preserved as many of the less important stages. Hand sections were stained in Methyl Green and Fuchsin, but the microtome sections were stained with Flemming's Triple Stain or Ehrlich's Haematoxylin.

The relatively large size of the oval-shaped embryo-sac and small size of the nuclei renders it difficult, and perhaps almost impossible, to obtain preparations in which the protoplasm is not somewhat strained. In some of the younger stages, in which the polar nuclei are only just in contact with one another, the sac is much smaller, and well filled with protoplasm; and in these the preservation is quite good. Later, the embryo-sac enlarges considerably, and the vacuoles

get so big that only a few slender strands of protoplasm bridge the central cavity.

The polar nuclei have generally completely fused before the entrance of the pollen-tube, but there is evidently a considerable amount of latitude in point of time, for I have found polar fusion in embryo-sacs of very different ages. The greater number of cases have been seen in sacs of the same age as that drawn in Fig. 1, or slightly older. Here the ovule is evidently quite young, for the integuments have not yet closed over the nucellus, while within the embryo-sac all the nuclei, even those of the antipodals, are in good condition, and all possess, as a rule, one central nucleolus. The antipodals are large pear-shaped cells and, at this period, very often contain two nuclei apiece produced by karyokinesis, but later, when the centre of the sac is occupied by one large definitive nucleus, the nuclei of each antipodal cell have run together to form a large irregular mass, containing many nucleoli. Frequently, however, the ovule is much older than this at the time of polar fusion, and in one instance the pollen-tube was seen in the micropyle at that period. This did not mean the early arrival of the pollen-tube, for the contents of the embryo-sac showed signs of age. Its cavity was immensely larger than that of the sac from which Fig. 1 was drawn, and its oosphere had increased in size while the synergids were somewhat degenerated; the antipodals also were bigger, and their centre occupied by a large deeply-stained nuclear mass. The coalescence of the polar nuclei is evidently taking place very late, for endosperm and embryos show most commonly in the other embryo-sacs of this ovary.

When the generative nuclei are extruded from the pollen-tube¹ they are very minute and stain heavily. Very often they are merely oblong, or lens-shaped, but sometimes they

¹ The swollen end of the pollen-tube is provided with a sharp point, evidently for boring through the nucellar tissue. I have seen the pollen-tube turn at right angles when passing through the nucellar tissue so that it entered the embryo-sac laterally.

are like small dumb-bells or may even have the form of a somewhat straight S. Their chromatic elements are so densely packed at this time that no structure can be made out. Although not able to follow it out with any certainty, I have always been strongly under the impression that it was the first nucleus that left the pollen-tube which fertilized the polar nuclei, for the polar fertilization is always much more advanced than that of the oosphere. I have read since that Professor Guignard finds this to be so in the Monocotyledons that he studied, and therefore it is extremely likely that it is the case in *Caltha* also. This may partly account for the fact that the process has been overlooked for so long. For when fertilization of the oosphere takes place, the polar fertilization may be too far advanced to be noticeable by any one not on the lookout for it.

By the time the vermiform generative nucleus has reached the middle of the sac, it has enlarged from an extremely small body to a nucleus of very considerable size, and its chromatic elements now show particularly clearly as small widely-scattered granules. The nucleus which fertilizes the oosphere, on the contrary, increases very little in size, and its chromatin still remains somewhat tightly packed, so that it is always very dark and shows merely a coarsely granular structure.

The result of the two fusions taking place at a slightly different time is of course that one rarely gets very good instances of both in the same sac. Where the ovum and male nucleus are actually coalescing, as shown in Fig. 2, the polar fertilization is in a late stage, so that there is only one end of the vermiform nucleus clearly distinguishable from the general polar mass (Figs. 3 and 4). In the sac from which Figs. 8 and 9 are taken, the male nucleus has only just reached the oosphere, and therefore, as one would expect, the vermiform nucleus, although tightly wound round the definitive nucleus, and indeed coalescing with it in parts, yet shows several of its sharp turns. In Figs. 6 and 7, however, we have a much better instance of the vermiform nucleus, for here it is only lightly applied to the definitive nucleus, and fusion has

not begun, so that we can follow its shape from end to end. It makes one large coil almost back upon itself, by means of which it rises on to the surface of the definitive nucleus.

I have seen a great many other sacs, in which it was perfectly evident that the second fertilization had taken place, but fusion had gone too far for one to be able to distinguish the vermiform nucleus with precision.

It will be noticed in the illustrations that I have given that the definitive nucleus does not present a rounded, even contour, but that it often appears as if the polar nuclei of which it is composed still retain their individuality to some extent. This is most marked in the fusion shown in Fig. 4, where the definitive nucleus still contains several nucleoli, and at one side the polar nuclei are quite separate. The definitive nuclei shown in Figs. 7 and 9 contain only one nucleolus, but there are deep indentations in their outlines which probably mark the boundaries of their component nuclei. This seems at first a little difficult to reconcile with the very numerous instances I have seen of one large, almost perfectly round, definitive nucleus, in sacs not yet fertilized. It is quite possible that some at any rate of these have passed the stage at which fertilization is possible, and that they then fully complete their earlier fusion. Some, however, certainly do unite completely, before fertilization, for I have seen a vermiform nucleus fusing with a single large definitive nucleus containing one central nucleolus.

The synergids also differ somewhat in their condition at the time of the entrance of the pollen-tube. Generally they seem fairly round and healthy, but very soon become disorganized. Sometimes they possess a somewhat broken outline, even when the pollen-tube first enters.

The antipodals, on the contrary, remain large and important-looking after fertilization has taken place, as Dr. Westermaier¹ has found to be the case in many members of the order Ranunculaceae.

¹ Dr. M. Westermaier, *Zur Embryologie der Phanerogamen, insbesondere über die sogenannten Antipoden.* Halle (Acad. Leop.), 1890.

The definitive nucleus divides by mitosis, and often forms a considerable amount of endosperm before the oosphere divides. The first wall formed in the latter is at right angles to the long axis of the sac.

All the practical work in connexion with this paper was done at the Royal College of Science last summer, but although I was convinced that a double fertilization, by means of vermiform nuclei from the pollen-tube, took place, I was unwilling to publish at the time, as I had not found a perfect and unmistakable fertilization of the oosphere by the male nucleus, in the same sac with the fertilization of the definitive nucleus. In examining my slides again this spring, however, I came across a perfectly satisfactory fusion of the oosphere and male nucleus previously overlooked (see Figs. 2 and 5). In the same sac is a sufficiently good polar fertilization, although only a small portion of the vermiform nucleus is to be seen, but, as I explained before, it seems unlikely that an equally felicitous stage of both will be found in the same sac.

My thanks are due to Miss Sargant for very kindly giving me her advice on some slides that I submitted to her.

Since writing this paper, I have seen Professor Nawaschin's recently issued account of double fertilization in Dicotyledons. As a representative of the Ranunculaceae he has examined *Delphinium*, and although not able to find the male generative nuclei free in the embryo-sac, he saw them as vermiform structures in the pollen-tube, and again when fusing with the nucleus of the oosphere and definitive nucleus respectively. In two respects *Delphinium* resembles *Caltha*: its polar nuclei unite before fertilization, and the fertilized ovum remains undivided for a considerable time. There are no figures of this plant.

Professor Nawaschin's paper is, however, more particularly concerned with two Composites, *Helianthus annuus* and *Rudbeckia speciosa*. In both these plants also, the polar nuclei coalesce before fertilization. In *Rudbeckia*, as I found in *Caltha*, the fusion of the one male generative nucleus with the

definitive nucleus *takes place before that of the other with the oosphere* (*l. c.*, Fig. 1, B).

The spermatozoids of *Rudbeckia*, and also of *Helianthus*, show the characteristic shape and granular structure extremely well. In *Helianthus* they are very long and twisted like a corkscrew, while they are shorter and thicker in *Rudbeckia*, but show their structure even better.

Nawaschin differs from Guignard in not having seen the spermatozoids of *Lilium* either homogeneous or with a spiral ribbon at any period. In *Caltha* they are so extremely small, when they first come from the pollen-tube, and take the stain so heavily, that I was not able to make out any structure whatever. They appeared homogeneous, but this may have been due to difficulties in observation. Later, when the one spermatozoid has reached the definitive nucleus, it is immensely swollen, and has a particularly light, spongy structure with minute scattered granules.

Professor Nawaschin has also made discoveries of the utmost importance in Orchids. These plants form no endosperm, and it is therefore of great theoretical significance to find that, even when the embryo has attained a considerable size, the three nuclei associated together in the centre of the sac have not coalesced. Two of these are the unfused polar nuclei, and there can be little doubt that the third is a spermatozoid, although it has lost its characteristic form (*l. c.*, Fig. 2).

Professor Nawaschin has always believed that the fusion of the second male generative nucleus with the definitive nucleus is a true fertilization, and he takes these results as in a great measure proving it. For our knowledge of the nature of true fertilization is so limited that at present we can say little more than that it is followed by division. And in this respect the result of the two fusions is identical.

I have also read Professor Guignard's paper on Tulips, in which he mentions that he has seen the double fertilization in some Dicotyledons, including Ranunculaceae.

EXPLANATION OF FIGURES IN PLATE XXX.

Illustrating Miss Thomas's paper on the Fertilization of *Caltha*.

Abbreviations: *n. of o.*, nucleus of the oosphere; *s.*, synergid; *g. n¹.*, first generative nucleus, that which fertilizes the oosphere; *g. n².*, second generative nucleus, that which fertilizes the definitive nucleus; *d. n.*, definitive nucleus; *ant.*, antipodal cell; *p¹* and *p²*, polar nuclei.

Fig. 1. Young embryo-sac with polar nuclei in the first stage of fusion (*p¹* and *p²*). × 450.

Fig. 2. Embryo-sac at the fertilization period. Nucleus of oosphere and first male generative nucleus, coalescing. The second generative nucleus is in a more advanced stage of fusion with the definitive nucleus. Antipodal cell displaced. × 245.

Fig. 3. Definitive nucleus with second generative nucleus from Fig. 2, under greater magnification. This section includes but a thin slice of these nuclei. They show more plainly in the next section. × 550.

Fig. 4. Next section of the definitive nucleus shown in Fig. 3. The polar nuclei of which it is composed are evidently not completely fused. It contains at least three nucleoli. The somewhat torn nucleolus to the extreme left seems to be a section of one of the others which has drifted there. It, however, prevents one from following out the vermiform generative nucleus which shows just below it. × 550.

Fig. 5. Upper part of embryo-sac shown in Fig. 2 under greater magnification. × 550.

Fig. 6. Portion of embryo-sac with much coiled second male generative nucleus fertilizing the definitive nucleus. The latter contains only one nucleolus, but has an irregular outline with one sharp constriction which may mark the point of fusion of its polar nuclei. × 200.

Fig. 7. Definitive group from Fig. 6, under greater magnification. × 550.

Fig. 8. Oosphere with male generative nucleus just touching its nucleus. × 550.

Fig. 9. Definitive group from same sac as furnished Fig. 8. The second generative nucleus is in an advanced stage of fusion with the definitive nucleus, but still shows its vermiform shape very distinctly. × 550.

Thomas, Ethel N. 1900. "Double fertilization in a dicotyledon—*Caltha palustris*." *Annals of botany* 14, 527–535.

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