CYTOLOGICAL STUDIES IN THE GENUS ULMUS.

II. The embryo sac and seed development in the common Dutch Elm.

by

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CHAPTER I.

Introduction.

As the investigation on the reduction division in the pollen mother cells of *U. hollandica belgica* Rehd. gave no clue to the causes of the sterility, it appeared necessary to study the reduction division in the embryo sac mother cells and the subsequent embryo development in this type.

In order to get sure affirmation of the general grower's consent, maintaining that under no circumstances germination of the seeds should take place, a large number of fruits were sown under different conditions, viz. *on* the earth, *under* the earth, about 1 or 2 cm deep, and upon wet filter paper in petri dishes. The fruits had been especially picked, as most of them, when ripe, contain only a dried-up seed, or even no seed at all. No seeds, however, germinated; after a time, the fruits appeared to have swollen, but in all cases the seed had totally disappeared. Going by a record from v. Tubeuf, that seeds of *U. campestris* have their optimal germination when sown as soon as they come from the tree, we also made these experiments with fruits immediately after their being gathered.

These negative results still gain in importance, if we take into consideration that in the year 1934 the Elms gave an exceptionnally large crop: a large number of the fruits held an apparently healthy embryo.

In order to bring the facts established by this study in a scheme more conveniently arranged, I give in the first place a description about material, methods and microscopical results, afterwards followed by a biological exposition and a discussion.

CHAPTER II.

Material and Methods.

Material was collected in the years 1933 and 1934 in Baarn and in several places in Amsterdam, where we could be certain to get the real *U. hollandica belgica* type: of late, many diseased trees have been replaced by a large-leaved type, presumably a hybrid of rather uncertain origin.

The year 1934 has already been mentioned as an exceptionnally favourable year for the fruiting, which fact probably was caused by the rather high temperatures and the absence of night-frosts during the end of March and the first part of April. The yield of some trees was so large, indeed, that, seen from a distance, one got the impression that the leaves had already broken out. The fruits themselves also developed conspicuously well, even those, which, when ripe, lacked any trace of a seed.

As the results of our studies during the year 1933 proved to be rather poor, it was planned out for the year 1934 to collect and fix systematically the fruits of a number of trees during the maturation period.

In 1933 some material had been collected of the following types:

U. hollandica belgica Rehd.
U. Pitteursii Kirch.
U. Wilsoniana Schneid.
U. vegeta Lindl. (*U. hollandica vegeta* Rehd.)
U. pumila pinnato-ramosa Henry.
U. montana superba Spaeth (*U. hollandica superba* Rehd.)

In the year 1934 we obtained fixations of:

U. Pitteursii Kirch.
U. montana fulva Hort.

and, moreover, a series of subsequent stages of *U. hollandica belgica*, by fixing fruits of this type almost every day of the maturation period. From *U. hollandica belgica* we studied the entire series, beginning by the opening of the anthers until the mature fruit, and that within a number of parallel series, obtained both from forced twigs and from fruits collected in the open.

In 1933 we made our fixations with Navashin's fluid exclusively, in 1934 Navashin, Carnoy with chloroform and Zenker were used; a number of botanists recommend the last-mentioned fixation as an excellent one in the case of embryological studies in plant material. The results obtained with Navashin always appeared to be satisfactory to very good; Carnoy with chloroform gave well

stainable material, the preparations, however, show much shrinkage and, consequently, considerable dislocations. Apparently, Zenker penetrates very difficultly and fixes rather coarsely: it satisfied least of all.

In 1934 we also tried to get results with sections made by hand; although, in many cases, these turned out well enough, it remained necessary to study entire series of sections; the right thickness was 15—20 μ. In this technique by far the best results were obtained, if it was followed by a cautious staining, e.g. with erythrosin-cyanin (Chamberlain), or gentian violet. A faster method with haematoxylin Hansen and subsequent counterstaining with light-green gave some results, but turned out to be too untransparent for thicker sections. In the end, exclusively haematoxylin Heidenhain was preferred, for sections 20 μ thick: if care is taken to a far-pushed differentiation, the results still surpass those obtained with other stains. We used frequently renewed haematoxylin solutions and differentiated only for about 5 minutes in iron-alum, followed by a prolonged stay in a saturated solution of picrid acid in water.

CHAPTER III.

Embryo sac development and fertilization.

The reduction division in the embryo sac mother cells passes off regularly and no irregularities could be observed: consequently we shall resign to give an elaborate description of it.

The result is the four-nuclear stage of the well-known type (fig. 1). All four nuclei keep alive: this also prevails for *U. Wilsoniana* and for *U. pumila pinnato-ramosa.* During this stage, the inner integument has grown out till about half-way the nucellus, the outer one has reached about a quarter of the nucellar height (fig. 2). Soon after the reduction division the cavity, wherein hitherto the nuclei were locked up closely, enlarges and thus gives rise to the embryo sac proper. The nuclei range in a more or less lozenge-shaped figure (fig. 3). This activity is followed by a period of rest during a couple of weeks. Then, apparently rather fast in succession, follow the divisions completing the mature embryo sac. These divisions seem to take place during the night: several times, figures like fig. 4 were met with; an actually dividing nucleus, however, never was seen. Obviously, the integuments are rather slow in growing up (compare fig. 4). The mature embryo sac contains: an egg-cell,

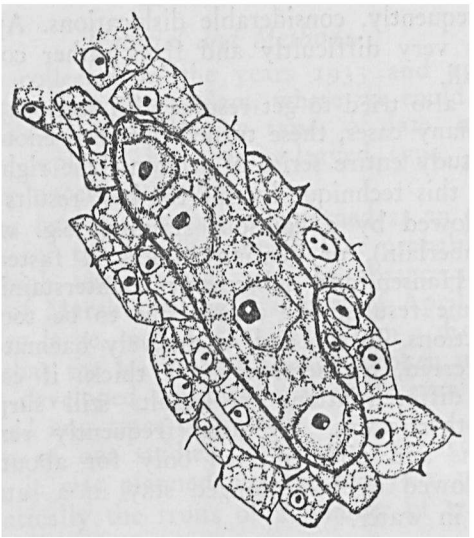

fig. 1

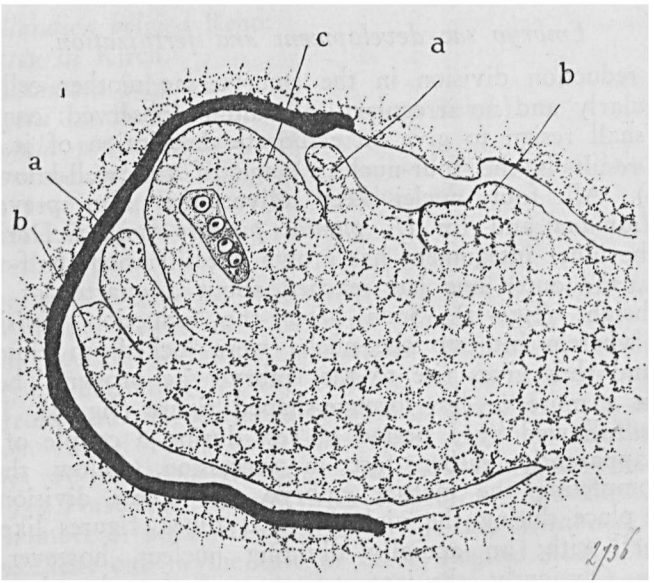

fig. 2

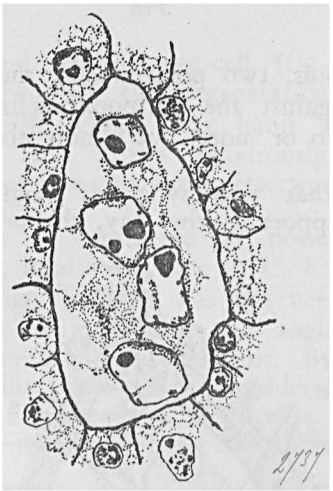

fig. 3

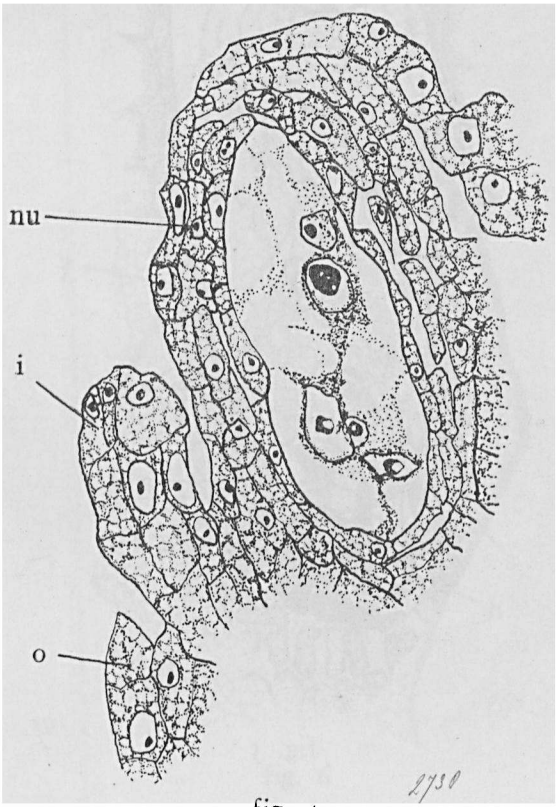

fig. 4

one or two synergids, two polar nuclei, the undermost of them ordinarily lying against the antipodal cells, which are present in a number of two or more, but almost always in a rudimental state. (fig. 5).

It is obvious that two synergids originate and this conjecture finds its support in the way, the protoplasm is arranged

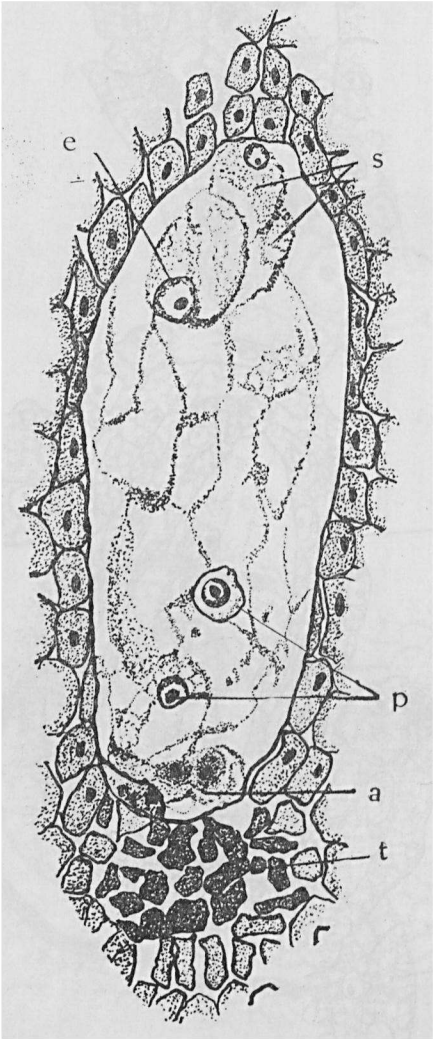

fig. 5

in the neighbourhood of the egg-cell (fig. 5). Even in the hardly mature embryo sac the degeneration of the antipodal cell could be observed. Hand over hand with the period of ripening the entire fruit grows vigourously and finally rises above the remains of the perianth: afterwards, we shall come back to this subject.

The genus Ulmus is considered as possessing an anatropic ovulum: this, indeed, is also the case in U. hollandica belgica, on the understanding that the bend has not been carried out completely, so that the ovulum makes an angle of about 45 degrees with the main axis of the fruit. By this bending the tissue in the funiculus undergoes a considerable stretching, with the consequence that it becomes lengthwise gaps, which might be considered as furrows, caused by the ingrowth of the pollen-tubes (fig. 6).

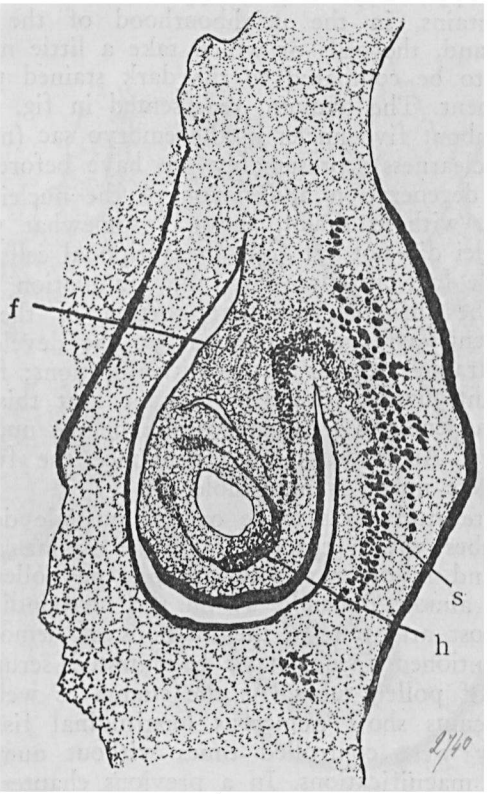

fig. 6

From the position of the embryo sac just mentioned and from the fact that the pollen tubes enter through the tissue and as least as possible pass the open spaces, it becomes clear that here the way of fertilization should be chalazogamic, just as in the Elm types already described. In very young stages already, the micropyle has disappeared entirely; only the arrangement of the cells in that part of the nucellus in longitudinal rows shows its earlier presence.

On the chalazal side a hypostasis is present (fig. 6), being part of the inner integument and made up of a tissue with lignin-containing cellwalls. Immediately above the hypostasis and lying against the embryo sac, a much more stainable tissue is seen, the cells being richer in protoplasmatic contents (fig. 6). Against the lignin-containing tissue and on the side of the chalazal vascular strand, we often perceived a losely built tissue of stone cells, which, apparently, may disappear later on. The outer integument contains, in the neighbourhood of the hypostasis, a very thin strand, the cells of which take a little more stain, but it is hardly to be compared to the dark stained portion of the inner integument. The ovulum, represented in fig. 6, contains at the moment about five nuclei in the embryo sac (not figured for the sake of clearness): presumably we have before us a mature stage, where degeneration and fusion of the nuclei already took place. Fig. 5, without doubt, shows a somewhat younger stage; the polar nuclei did not yet fuse, the antipodal cells are in degeneration, which fact appears from the granulation of the protoplasm and the shrinkage in that portion of the embryo sac. Fig. 4 represents a stage in the embryo sac development of *U. Wilsoniana*, drawn after two consecutive sections; from the position of the integuments, we must assume that this is a case of a rather young embryo sec, the position of the nuclei supporting this conjecture. An endeavour to denounce the future function of each of these nuclei is impossible.

An elaborate study was made on the possible development of the pollen tubes within the tissue of the stigma and the style. As, in this and in other related groups, the pollen tubes sometimes can be almost invisible within the surrounding tissues, we paid the utmost attention to the methods of demonstration, and the above-mentioned tissues have been tested scrupulously upon the presence of pollen tubes. As the stigma as well as the style and the funiculus show numerous longitudinal fissures (figs. 6, 7 & 8), they were controlled times without number with the help of large magnifications. In a previous chapter the different

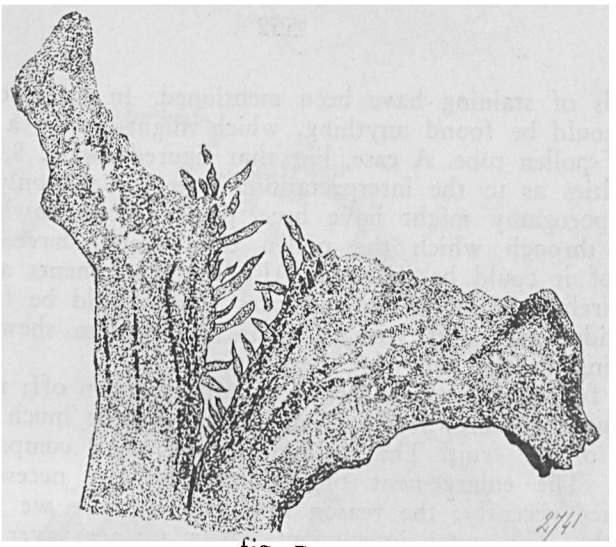

fig. 7

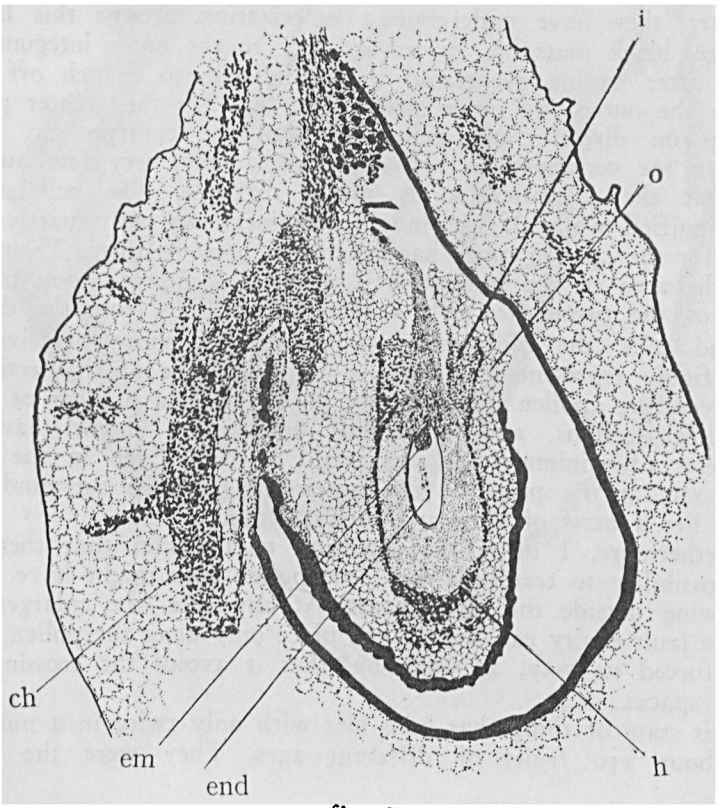

fig. 8

methods of staining have been mentioned. In no case, however, there could be found anything, which might justify a denomination of pollen tube. A case, like that figured in fig. 8, gave some difficulties as to the interpretation: here, for the only time met with, porogamy might have been possible, but nowhere in the tissue, through which the pollen tube should have entered, a trace of it could be detected. Also the integuments appeared to be entirely free in all the instances which could be faced, untill two slides were met with, wherein the ovulum shew an image according to the following description.

The funiculus has been drawn out, partly torn off; the ovulum, consequently, hangs freely from a stalk in the much enlargened cavity of the fruit. The ovulum is very small compared to the cavity. The enlargement of the cavity must necessarily have happened recently: the reason of this conjecture we will discuss presently. The outer integument has a surface layer of cubical cells which, at the sides, are dying off; at the undermost, chalazal part, they have undergone a suberization. Across this layer a huge, black mass has forced its way to the outer integument, then, after having penetrated this wall, tries to branch off between the outer and inner integument, but, for the greater part, carries on directly and penetrates into the embryo sac. The embryo sac contained in both cases seen, only very few nuclei, so that the identification is rather difficult. The cells with the lignified walls of the inner integument are only partly intact, for the „pollen tube" has penetrated between them. Throughout the area of the integuments, where the entering took place, part of the tissue died off, a fact recognizable from the dark-stained cells, the contents of which are no more perceivable. Our figure represents the state of things in one of the sections: however, the „pollen tube" can be perceived in a number of surrounding sections, and, from our calculation, should have a diameter of minimally 45, maximally 75 μ ! Even in the embryo sac, at the place of the penetration and its surroundings, there lies a mass of torn and died-off tissue.

Furthermore, I draw the attention to the fact that there is no possibility to trace the way, the pollen tube might have been following outside the integuments. At any rate, the enlargement of the fruit cavity can have taken place only after the pollen tube had forced its way, as we know that it avoids the crossing of open spaces.

This state of things has been met with only twice in a number of about 250 fruits of different ages. They were the only

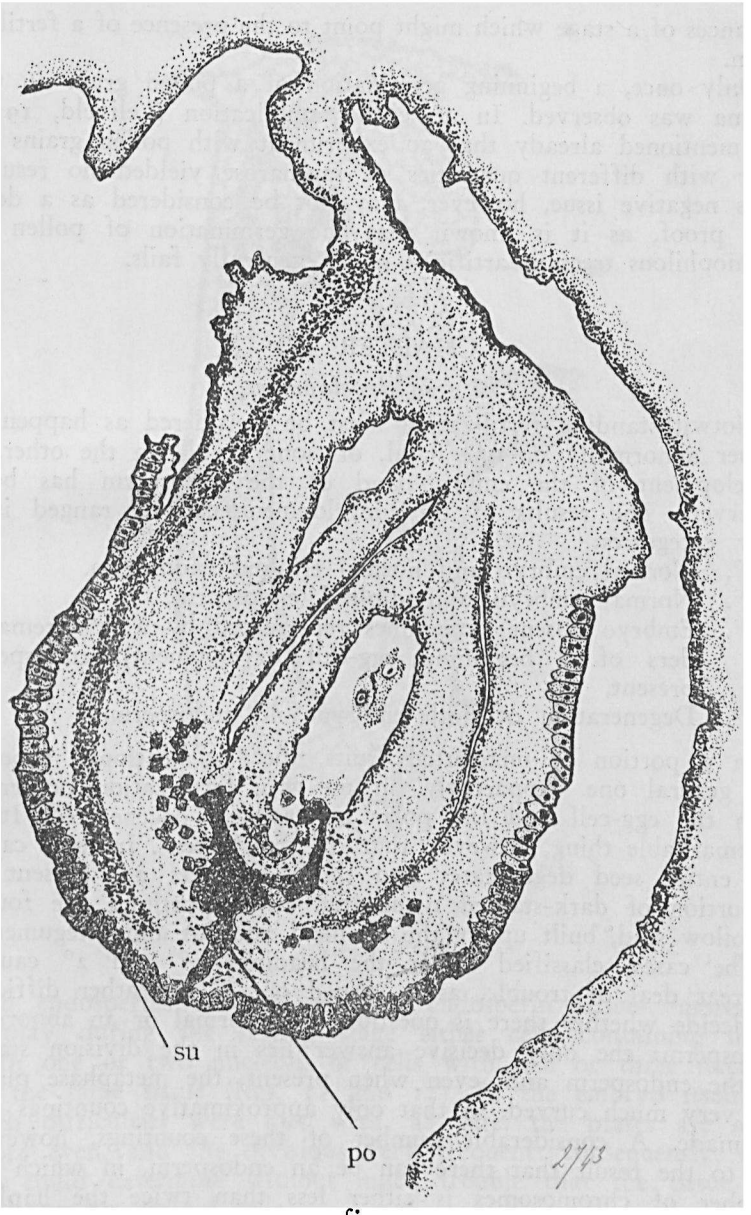

fig. 9

instances of a stage which might point to the presence of a fertilization.

Only once, a beginning germination of a pollen grain on the stigma was observed. In an earlier publication (Leliveld, 1933) we mentioned already that an experiment with pollen grains on agar with different quantities of saccharose yielded no results. This negative issue, however, may not be considered as a definite proof, as it is known that the germination of pollen of anemophilous trees in artificial media generally fails.

CHAPTER IV.

The seed development.

Notwithstanding fertilization must be considered as happening rather abnormally on one hand, or none at all on the other, a development of the embryo and of the endosperm has been observed. The results of this development can be ranged into four categories:

1°. Normal embryo and normal triploid endosperm,
2°. Normal embryo and diploid endosperm,
3°. Embryo absent (sometimes represented by a few remainders of a beginning egg-cell development), endosperm present,
4°. Degeneration of both embryo and endosperm.

In a portion of unassorted fruits, the last-mentioned type is the general one. Apparently, in no way fertilization occurred; both the egg-cell and the polar nuclei have degenerated. It is a remarkable thing, however, that the fruit ripens. In most cases, the entire seed degenerates and the remainders are present as a portion of dark-stained tissue (fig. 10). Sometimes, we found a hollow seed, built up by the shrinked nucellus and integuments.

The cases, classified under the headings 1° and 2° caused a great deal of trouble, as it turned out to be rather difficult to decide whether there is question of a normal or an abnormal endosperm: the only decisive answer lies in the division stages of the endosperm and, even when present, the metaphase plates are very much curved, so that only approximative countings can be made. A considerable number of these countings, however, led to the result that there can be an endosperm, in which the number of chromosomes is either less than twice the haploid number (the latter being fourteen chromosomes), or less than thrice that number: these results indicate that diploid as well as trip-

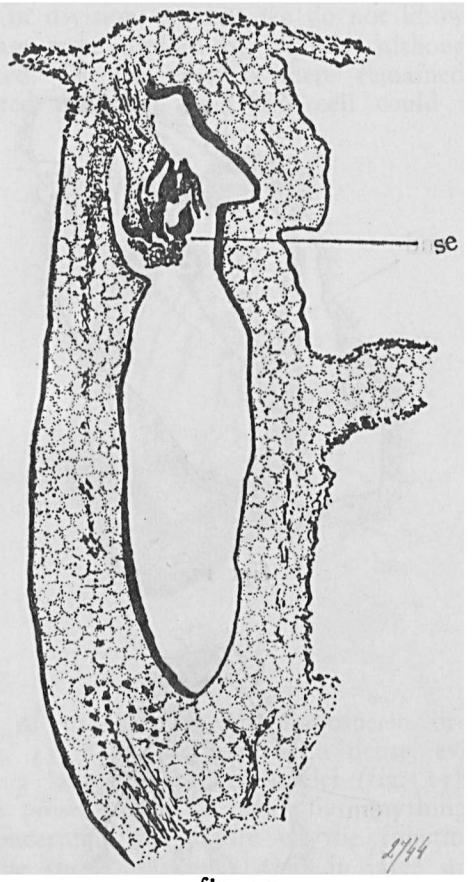

fig. 10

loid endosperms may occur. The endosperm tissues, moreover, display during the resting stage either cells containing nuclei with one or two nucleoli, or cells with one or three nucleoli, in the same tissue (figs. 11 and 12). In the embryo itself, no such difficulties were met with, as here, the plates are much more even, and the divisions very frequent: consequently, here, we could determine without much trouble that the tissue was built up by diploid cells.

Far from infrequent was the case that only the endosperm had developed: infortunately, in none of these instances the

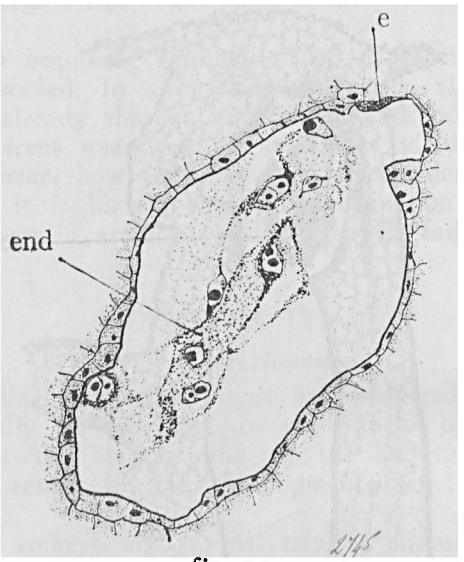

fig. 11

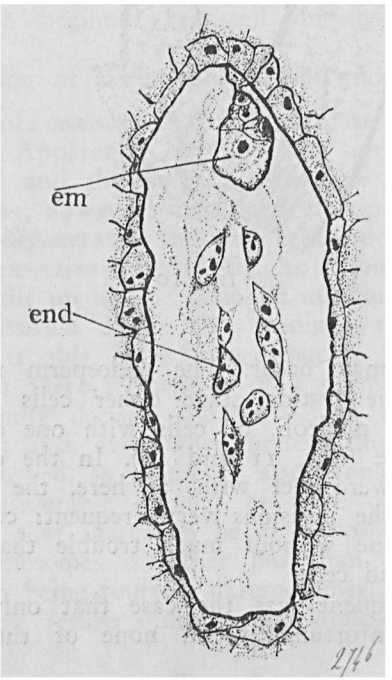

fig. 12

endosperm was in division, so that we do not know, whether the cells in that case were diploid or triploid, although the former may be expected. Of the embryo there remained, at most, a single degenerated cell, but even this cell could fail (fig. 13).

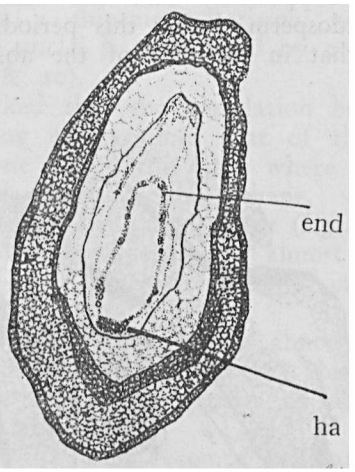

fig. 13

At the side of the chalaza the endosperm broadens into a haustorium (fig. 13, 14), composed of a dense, even protoplasm which contains a large number of nuclei (fig. 15). It is hardly possible, in the present case, to make out anything certain from preparations concerning the nature or the function of this organ. It could be stated, however, that in later stages the haustorium gradually becomes smaller, together with the disappearance of the endosperm, a fact coming into discussion later on (fig. 20). With the inner integument, the haustorium is in connection only by means of some very thin protoplasma strands.

During the four cell stage of the embryo, the endosperm cells ly still scattered throughout the cavity of the embryo sac (fig. 12). The divisions in the young embryo evidently pass away very fastly; we rarely met with stages between those made up by four cells and the embryos figured in figs. 14 and 16, both these cases representing an embryo of about 64 cells. The endosperm, by this time, is parietal and consists to a layer of protoplasm in which the nuclei are imbedded in a single row. At the top of the embryo sac the protoplasm of the endo-

sperm layer introverts, thus giving rise to an indenture in which the embryo lies imbedded.

The close contact between the growing embryo and the surrounding endosperm throughout its development (figs. 14, 16—19, 21) justifies the supposition that the embryo might be feeding upon the endosperm during this period. It is, moreover, a remarkable fact that in the case of the absence of an embryo

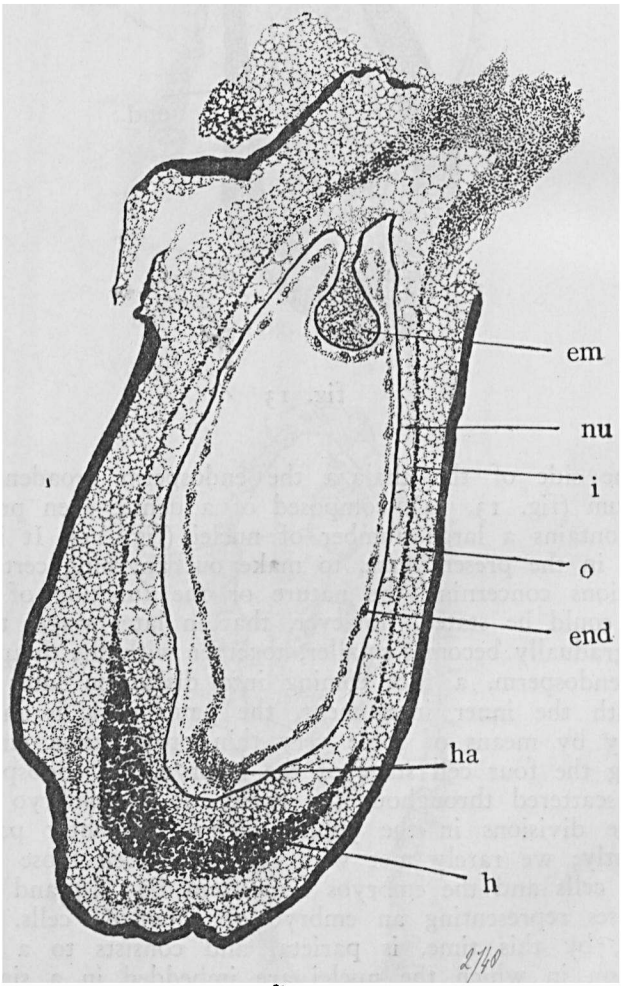

fig. 14

(fig. 13) the endosperm occupies a much smaller part of the embryo sac.

During a considerable time of the development the divisions in the endosperm only tend to increase the number of nuclei within the wall layer (fig. 17). Only afterwards the cavity becomes filled up by a thin-meshed tissue, now made up by cells, as each of the nuclei is surrounded by protoplasm confined by a wall (fig. 19 & 20).

It may be remarked that the correlation between the development of the young embryo and that of the endosperm can be highly lacking: one meets with cases where the embryo shows already the typical dicotyledonous shape, whilst the endosperm is only represented by a wall layer (figs. 17, 18); in other instances, however, the endosperm fills almost the entire cavity and the embryo is still far more behind in its development (fig. 19).

In this relation we may point to an abnormal case (fig. 21), where a double embryo came into being, but, evidently there is a large difference between the stages of development of the embryos, marked *a* and *b*.

Soon after the endosperm becomes cellular, a degeneration process sets in, causing the gradual thinning out of the endosperm; this tissue, indeed, never filled up the entire cavity, but always left a spare room in the lower part of the embryo sac (fig. 20). It is a little above the open space just mentioned that the vanishing of the endosperm starts. It is a well-known fact that the ripe seeds of the Elm species in general do not contain any more endosperm (c.f. Rehder, p. 183).

CHAPTER V.

Abnormal seed development.

In his article on *Ulmus americana*, Shattuck mentions a few cases of abnormal embryo development observed during his study. Obviously not infrequent was the presence of an antipodal egg besides the normal egg-cell. We met with this case only once (fig. 22). If we take into consideration the fact that in U. americana the antipodal nuclei subsist for a much longer time we need not be surprized at that. In the case of a development of an antipodal egg in *U. hollandica belgica* we, apparently meet with a doubly abnormal case for the species:

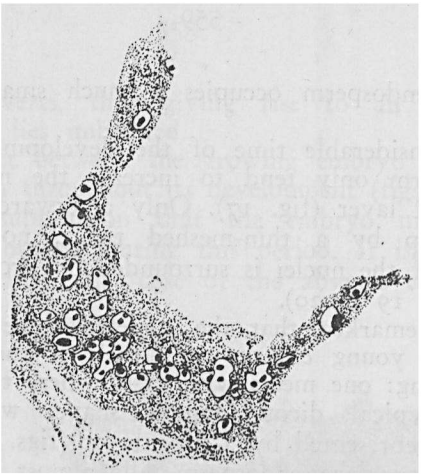

fig. 15

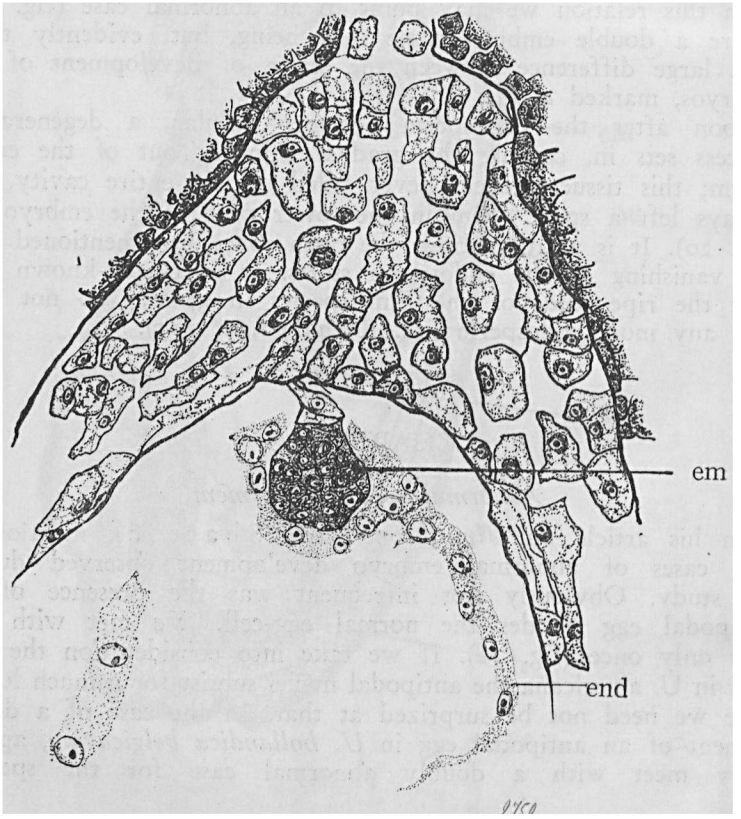

fig. 16

1° the subsisting of an antipodal cell and 2° the following development of the said cell into an embryo.

Another abnormality has been figured in fig. 21. Here, one of the synergids must have grown out with the result that two embryos of a normal shape are lying next to each other at the top of the embryo sac. In this stage it is no more possible to settle which of the two embryos has developed from the egg-cell. In fig. 21a we figured the eldest of the two embryos, which, already possesses the normal dicotyledonous shape; the younger one which could be discerned in its full development in another section of the same slide, showed a radicula, but not a trace of bilobedness and, what would be the consequence of this stage of development, of a plumula.

Fig. 23 shows an abnormality affecting the entire ovulum. Obviously, the ovulum has endeavoured to reach its normal more or less anatropic position, but at the same time the axis re-bent and the result is, nevertheless, an atropic ovulum. Also this abnormal case has only once been met with.

CHAPTER VI.

The correlation between the pollen- and embryo sac development.

Navashin, in 1894 reports for *U. effusa* that the pollen and the embryo sac are mature at almost the same moment. An immediate fertilization, consequently, may happen and, indeed, has been stated by him.

As soon as in U. hollandica belgica the flowers have shed their pollen, one sees the fruit grow out. The samara, hitherto almost hidden between the perianth, grows above them. At first, we thought this to be a consequence of the fertilization and therefore numerous stages were fixed and sectioned. In none of them, however, a trace of a pollen tube could be demonstrated. It appeared, indeed, that at the time of the shedding the embryo sac was still in a primitive stage and although the number of nuclei was varying between four and eight, none of these stages could be said to have reached maturity. But, in this period there takes place a strong outgrowth of the integuments which at the time of the pollen maturation were rather rudimentary and remained in that condition for a time afterwards (fig. 4). The outgrowth causes the fruit to push above the other parts of the flower. Only about two or three weeks after the shedding of the pollen the embryo sac may be said to be full-grown.

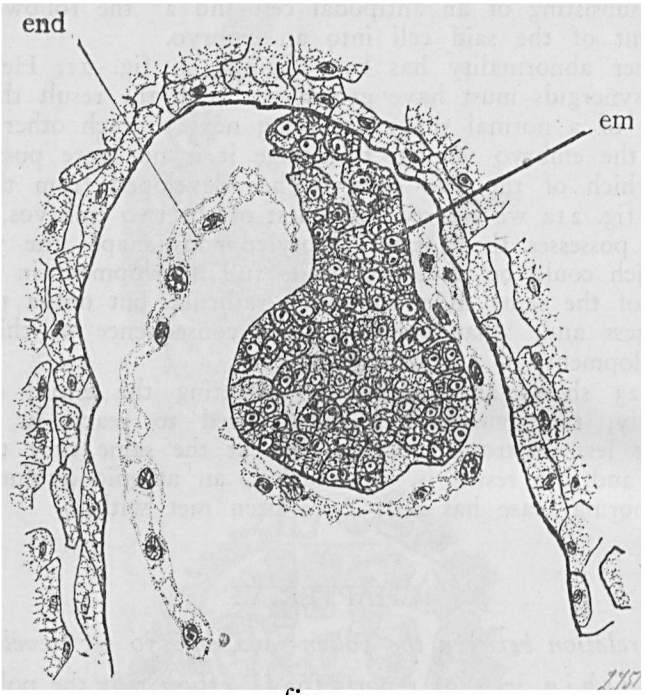

fig. 17

Now, all the trees belonging to the hollandica belgica-type show the peculiarity that they flower simultaneously and, consequently, the shedding of the pollen comes to an end in about two or three days: this, obviously, must be seen in connection with the fact that all the material runs back to the same parent.

The chance that, during the maturity of the embryo sac, pollen of the same type should be present upon the stigma, consequently, grows very small: indeed, only very seldom we came across pollen on the stigmata. Our histological research also eliminated the possibility of a very slow and prolonged growth of the pollen tubes within the tissues, — a fact sometimes observed in the group of the Amentiflorae and also reported for Celtis (Modilewsky).

Almost with certainty we may presume that there is no question of a fertilization by the pollen originating from the same type. Whether such a fertilization would be successful, remains an open question appearing to be only solvable by the experiment.

The sporadic fertilizations must needs have been caused by pollen originated from other Elm types. We attempted to decide, whether there exists a difference between the pollen grains of U. hollandica belgica and those actually found on the stigmata. No difference, however, could be observed between them (fig. 24).

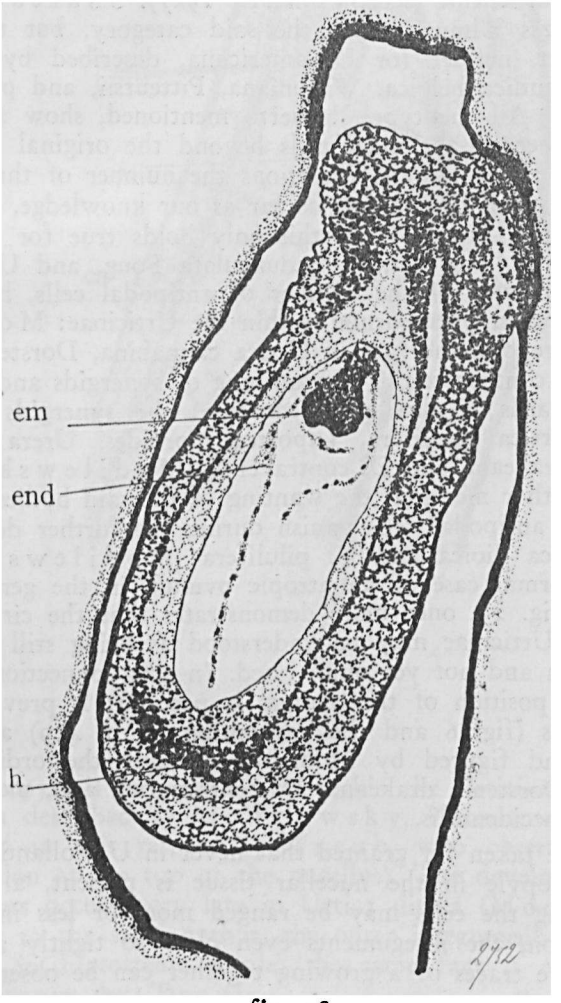

fig. 18

CHAPTER VII.

Discussion.

Several points in the preceding description need a comparison with the data already known from other investigators.

The embryo sac development reminds of that, generally known as the Lilium-scheme (see Schnarf 1927): Shattuck (1905), indeed, ranges Ulmus within the said category, but this is not quite correct, neither for U. americana, described by him, nor for U. hollandica belgica, Wilsoniana, Pitteursii, and pumila pinnato-ramosa. All the types, hitherto mentioned, show an increase of the number of antipodal cells bevond the original number of three. Navashin (1898) mentions the number of three for the genera Celtis and Ulmus, but as far as our knowledge, at present, goes about the genus Ulmus, this only holds true for the species investigated by him, viz. U. pedunculata Foug. and U. montana With. An increase of the number of antipodal cells, however, is a not uncommon phenomenon within the Urticinae: Modilewsky (1908) reports the fact in Urtica cannabina, Dorstenia drakeana and D. contrayerva. The vanishing of synergids and antipodal cells afterwards is also quite common: the synergids disappear early in Urtica pilulifera, Laportea moroides, Urera baccifera, Dorstenia drakeana and D. contrayerva (Modilewsky), whilst the same author mentions the wanting of the said bodies in Urtica dioica. The antipodal cells vanish during the further development within Urtica dioica and U. pilulifera (Modilewsky).

The abnormal case of an atropic ovulum in the genus Ulmus, figured in fig. 23, once more demonstrates that the circumstances within the Urticinae may be understood as being still in a state of transition and not yet quite fixed. In this connection, also the „half-way" position of the ovulum is peculiar: it prevails in the genus Ulmus (fig. 6 and the description on p. 459) and is also described and figured by Modilewsky, being the ordinary condition for Dorstenia drakeana, D. contrayerva, and, more or less, for Celtis occidentalis.

It may be taken for granted that never in U. hollandica belgica a real micropyle in the nucellar tissue is present, although, in the beginning the cells may be ranged more or less in a double row: later on, the integuments even close so tightly at the top that no more traces of a growing together can be observed. Only once the nucellus was seen growing out of the integuments in the direction of the style (fig. 8); this fact reminds of the description given by Modilewsky for Laportea moroides. The absence

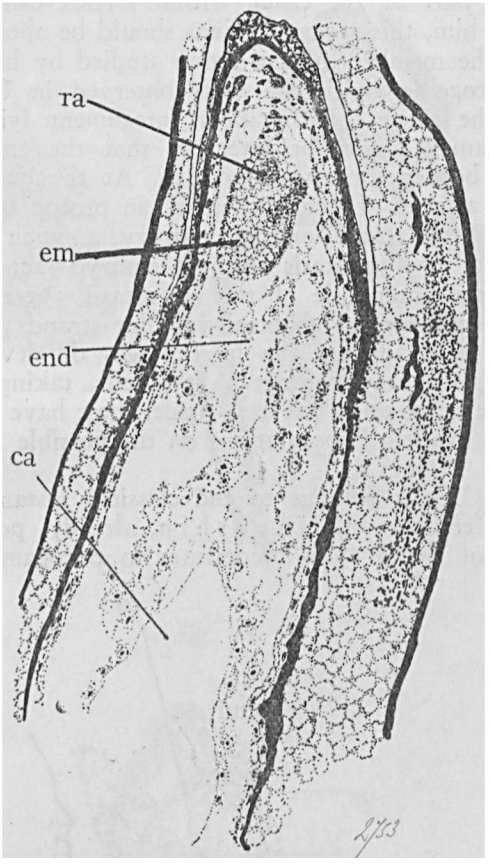

fig. 19

of a micropyle is rather common within the Urticinae (e.g. Urtica cannabina described by Modilewsky, Elatostema acuminatum mentioned in this respect by Treub, who observed a strong sclerification at the top of the nucellus). The development of the integuments occurs very late in Urtica dioica (Modilewsky); according to the same author, the outer integument remains only half-way in Elatostema sessile; the same fact is mentioned for E. acuminatum by Treub.

The hypostasis is a part of the outer integument, very common in the Urticinae: the description, however, given by Modilews-

k y for this part of the tissue within Urtica cannabina — and according to him, this state of things should be about the ordinary one for all the members of the group studied by him — does not entirely fit together with the facts observed in Ulmus spec. In his objects the tissues of the inner integument lying against the hypostasis vanish sooner or later, so that the embryo sac then immediately borders on the hypostasis. At the outer side of the hypostasis is a layer of small cells, rich in protoplasmatic contents. Within Ulmus we found, on the contrary, a much more stainable tissue between the hypostasis and the embryo sac: and this tissue persists. At the outer side of the hypostasis a great many stone cells are present near the chalazal vascular strands, but these seem to disappear ofter during the later stages of development. The rest of the tissue may contain a few cells, taking a little more stain than the other ones, but as a tissue, these have no importance. We prefer to evade all speculations on the possible function of the hypostasis.

The genus Ulmus belongs to the classical instances of a non-porogametic fertilization. N a v a s h i n, already, pointed out that the position of the ovulum predestines to porogamy. The differ-

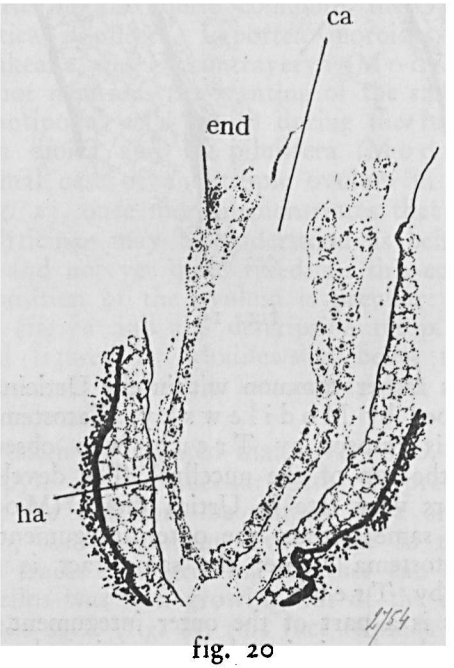

fig. 20

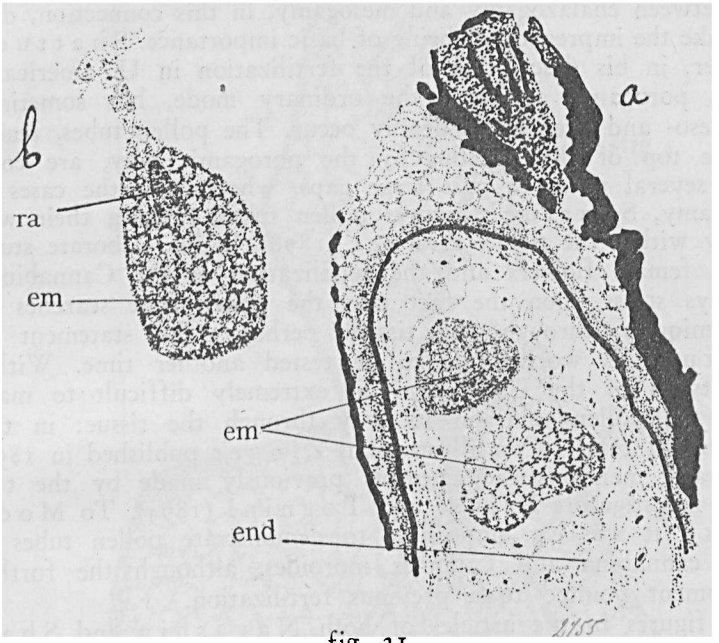

fig. 21

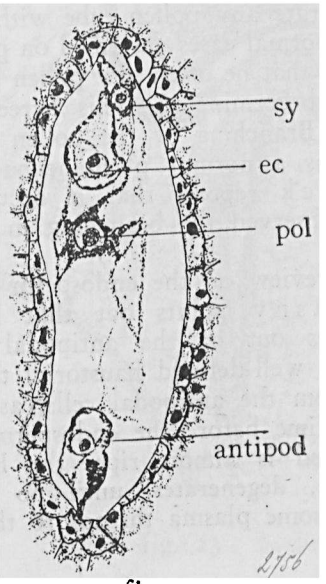

fig. 22

ence between chalazogamy and mesogamy, in this connection, does not make the impression of being of basic importance. Shattuck, however, in his description of the fertilization in U. americana, reports porogamy as being the ordinary mode, but sometimes also meso- and even chalazogamy occur. The pollen tubes, reaching the top of the nucellus by the porogamic way, are compelled several times to pass large gaps, whereas, in the cases of aporogamy, Shattuck figures pollen tubes, making their way entirely within the tissue. Zinger (1898), in his elaborate study on the female flowers and the fertilization in the Cannabinae, also lays stress upon the fact that the pollen tube searches its way uniquely through the tissue: perhaps, the statement of Shattuck is worth wile to be tested another time. Within many types of the Urticinae it is extremely difficult to make visible the pollen tube on its way through the tissue: in this connection I refer to the illustrations Zinger published in 1898: he already mentions the attempts previously made by the two Italian investigators Briosi and Tognini (1894). To Modilewsky it was e.g. impossible to demonstrate pollen tubes in Urtica cannabina and Laportea moroides, although the further development pointed to a previous fertilization.

The figures in the articles of both Navashin and Shattuck gave the impression that the pollen tubes in the Ulmoideae are not so difficult to observe. In spite of all our efforts we were not able to demonstrate any pollen tube within the tissue, except for the two very abnormal cases described on p. 462 Modilewsky relates for Celtis that he often saw pollen grains on the stigma, but these never had germinated. This agrees with the circumstances seen by us. Branching of the pollen tubes on their way through the tissue is a normal phenomenon according to Navashin. Shattuck reports that it occurs in the cases of belated tubes. We observed the branching in both cases of "fertilization".

In her elaborate review on the endosperm and the haustorium Jacobsson—Stiasny points out that in many cases the haustorium originates out of the antipodal cells: although in Ulmus there exists a well-defined haustorial tissue, it is sure that it does not arise from the antipodal cells, as the last ones have degenerated a long time before the endosperm starts its development. When the seed is almost ripe, the haustorium, together with the endosperm, degenerates, until no more than a thin layer, connected by some plasma threads to the inner integument, is left.

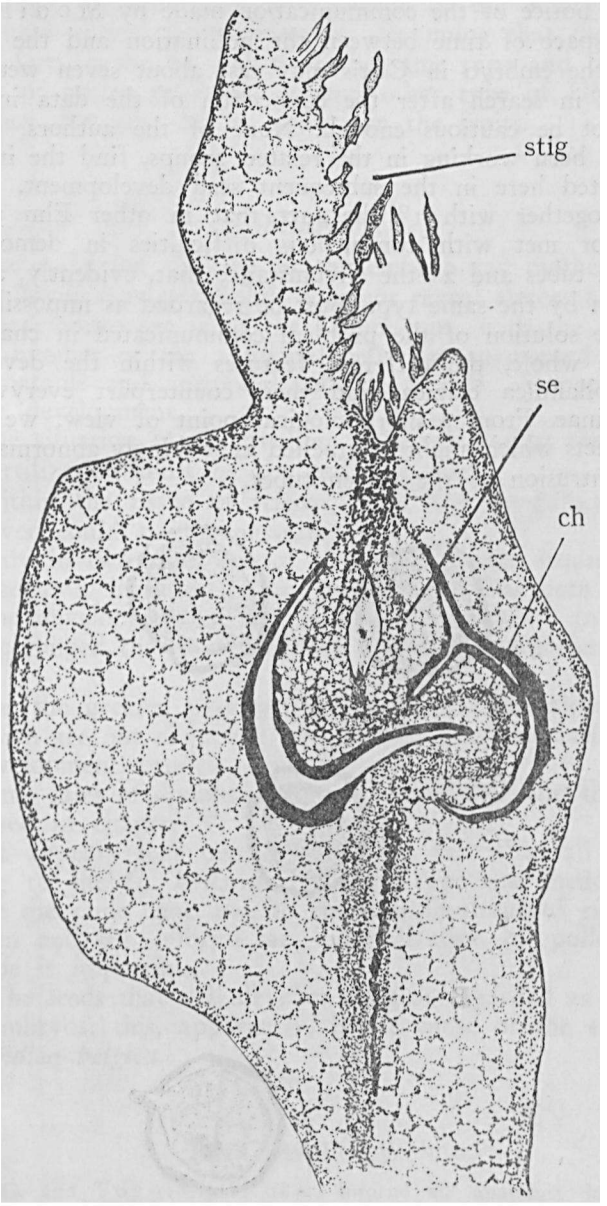

fig. 23

Taking notice of the communication made by Modilewsky that the space of time between the pollination and the development of the embryo in Celtis may last about seven weeks, it is clear that in search after the connection of the data in Ulmus, one cannot be cautious enough. None of the authors, however, who have been working in the related groups, find the irregularities reported here in the subsequent seed development.

This, together with 1° the fact that in other Elm types no investigator met with conspicuous difficulties in demonstrating the pollen tubes and 2° the circumstance that, evidently, a normal pollination by the same type must be regarded as impossible, have led to the solution of the problem communicated in chapter VI.

On the whole, the different features within the development of U. hollandica belgica find their counterpart everywhere in the Urticinae. From a morphological point of view, we did not observe facts which might be labelled as strikingly abnormal, except for the intrusion of the pollen tubes.

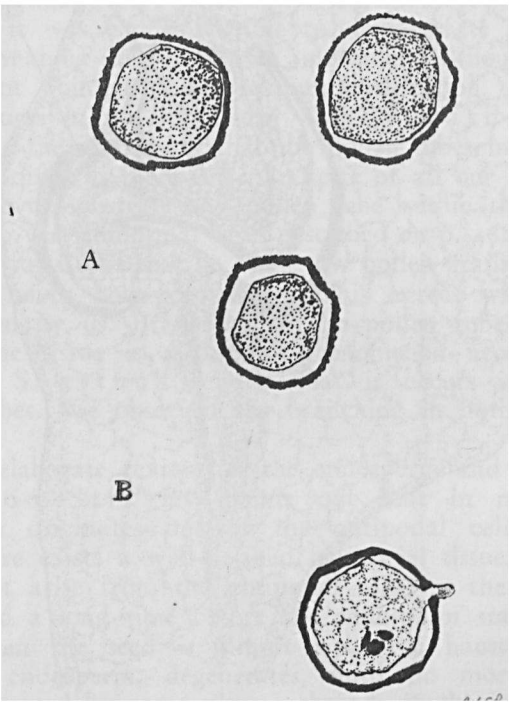

fig. 24

Concerning the abnormal development of the embryo we believe to have solved the problem by presuming that the impossibility of fertilization by pollen of the same type and the incompatibility of pollen produced by any other type of Ulmus, may be together the cause of the failure in the fruits.

SUMMARY.

1. The reduction division in the embryo sac mother cells of *Ulmus hollandica belgica* Rehd. does not reveal abnormal features.

2. The embryo sac is of the Lilium-type, with the restriction that the number of the antipodal cells has augmented.

3. Synergids and antipodal cells, as a rule, disappear during the later development.

4. The situation of the ovulum makes clear that the chalazogamic fertilization must be the ordinary one.

5. Within the tissue of stigma, style and integuments pollen tubes never could be demonstrated.

6. Only in two cases out of about 250 ovules studied a kind of chalazogamic intrusion by a "pollen tube" has been observed.

7. The development of the seed may take place in different ways: we ranged the cases observed into four categories (Chapter IV).

8. For the greater part the seeds show abnormalities and in those which are not abnormal, yet a lacking in correlation during their development can be observed.

9. Some cases of double embryos are described and their origin is discussed in chapter V.

10. It appears that owing to 1° the fact that all the trees belonging to the *U. hollandica belgica* type are shedding their pollen at the same time and 2° the non-simultaneous ripening of the pollen and the embryo sac, a fertilization by pollen of the same type is impossible.

11. The seeds that still develop must be regarded as possessing hybrid embryos: this, apparently, is the cause of the sterility in *U. hollandica belgica.*

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EXPLANATION OF THE FIGURES.

Fig. 1. Four-nucleate stage after the reduction division.

Fig. 2. Ovulum on its way to the anatropic position. a: Inner integuments, b: outer integuments, c: nucellus.

Fig. 3. Four nuclei in the future embryo sac in a zig-zag position.

Fig. 4. Young embryo sac with 5 nuclei. The integuments not yet fullgrown. Nu: nucellus, i: inner integument, o: outer integument.

Fig. 5. Mature embryo sac. E: egg-cell, s: synergids, p: polar nuclei, a: antipodal cells, t: hypostase tissue.

Fig. 6. Ovulum surrounded by the fruit wall. For the sake of clearness, the contents of the embryo sac have been omitted. f: fissures in the funiculus, s: stone cells in the chalazal region, h: hypostasis.

Fig. 7. Stigma, showing the numerous gaps and fissures in its tissue.

Fig. 8. Ovulum surrounded by the fruit wall, the embryo (em) and the endosperm (end) already in development. The nucellus shows en outgrowth in the direction of the style. ch: chalaza, i: inner, o: outer integument, h: hypostasis.

Fig. 9. „Pollen tube" (po) entering the embryo sac, after having passed the surrounding tissue. See text. Su: suberized tissue.

Fig. 10. Fruit with degenerated seed (se).

Fig. 11. Embryo sac with endosperm (end), containing nuclei with either one or two nucleoli, egg-cell (e) degenerated.

Fig. 12. Embryo sac with endosperm (end), containing nuclei with either one or three nucleoli, a small embryo (em) developing.

Fig. 13. Seed containing no embryo, and a very scanty endosperm (end). ha: Haustorium.

Fig. 14. Seed with normally developed embryo (em) and endosperm (end). ha: Haustorium, nu: Nucellus, i: inner integument, o: outer integument, h: hypostasis.

Fig. 15. Haustorium of fig. 14 magnified, showing a dense plasmatic mass with a great many nuclei in it, each containing one or two nucleoli.

Fig. 16. Embryo of about 64 cells (em), closely encircled by the endosperm (end).

Fig. 17. The embryo tends to get its dicotyledonous shape. Endosperm in division.

Fig. 18. The embryo in a slightly advanced stage. The torn-off endosperm does not show any advance as compared to figs. 16 and 17.

Fig. 19. Embryo with radicle (ra), more or less dicotyledonous; endosperm filling almost the entire seed, except for a cavity at the lower end (ca).

Fig. 20. Same seed as fig. 19: the lower part, showing the cavity (ca) and the quickly degenerating haustorial tissue (ha).

Fig. 21. Seed containing a double embryo: a. one embryo with typical dicotyledonous shape and part of the other one, b. the other embryo, not yet differentiated into a dicotyledonous type, but still possessing its radicle.

Fig. 22. Embryo sac with antipodal embryo (antipod), sy: synergid, ec: egg-cell, pol: polar nucleus.

Fig. 23. Ovulum, after having tried to reach its anatropic position, re-bent, and resulting in an atropic position. stig.: stigma, se: ovulum, ch: chalaza.

Fig. 24. A. Pollen grains of U. hollandica belgica at the time of shedding.
B. Pollen grain as found on the stigma of a fruit containing a mature ovule, and actually pushing a pollen tube. Both drawn under the same magnification.

All drawings have been done with the aid of a Zeiss camera lucida at table height, except for fig. 10 which has largely been drawn by hand.

Figs. 1, 3, 4, have been observed with a Zeiss apochromatic imm. 1.5 mm. with Zeiss comp. oc. 5 x.

Figs. 2, 5, 11, 12, 15, 16, 22, have been observed with a Zeiss D- lens (40 x), together with an ocular 5 x.

Figs. 6, 7, 8, 13, 14, 17, 18, 19, 20, 21, 23, have been observed with a Zeiss A- lens (8 x), together with an ocular 5 x. Fig. 9, also was observed with an A, together with an eye-piece 10 x.

Fig. 24 was observed with a Zeiss immersion 1/12, together with an eye-piece 5 x.