

XI. *Contributions to the Embryology of the Amentiferæ.*—PART I. By MARGARET BENSON, *B.Sc.*, *Marion Kennedy Student, Newnham College, Cambridge.* (*From the Botanical Laboratories of University College, London, and of the University of Cambridge.*) (*Communicated by Prof. F. W. OLIVER, M.A., D.Sc., F.L.S.*)

(Plates LXVII.—LXXII.)

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IN November 1891 Professor F. W. Oliver suggested that I should investigate the embryology of the British Amentiferæ. He had on several occasions expressed his regret that such an interesting group of plants should be comparatively neglected by the botanist, and on the appearance of Treub's magnificent treatise on *Casuarina** the need became more urgent. The work has been throughout conducted under his guidance, although it has been latterly carried on in a Research room kindly allotted to me in the University Botanical Laboratory, Cambridge, by Mr. F. Darwin.

The onerous nature of the investigation will be understood if one recalls the long list of genera which are grouped together under the somewhat elastic term Amentiferæ, which I will use in the present paper as including the Cupuliferæ, Corylcæ, Betulineæ, Juglandaceæ, and Salicineæ.

There was little material in hand; but during the spring and summer of 1892 I collected complete series of the female flowers of representative species of eleven genera, and of many I have been at no little trouble to obtain parallel series of different species.

The flowers were always preserved in absolute alcohol, although occasionally in picric or chromic acid also. There is but little literature to guide one as to the date of the important stages, even if such particulars could be of much use in a climate like ours; and the styles having withered often for months, the result was that very frequent gatherings had to be made long before the actual processes occurred which were sought. Fortunately it is tolerably easy to obtain good results by the examination of fresh material, although in the most critical periods the difficulty was increased by the large number of ovules that normally abort, for repeated preparations had to be made before one could rely on the ovules being such as were continuing their development. The result has been that, although over two hundred bottles of material were collected, and unremitting attention was given from March until September, I am still obliged to rely on this year's material (*i. e.* 1893) for a stage of development in two or three cases. Lastly, the flowers belonging, for the most part, to our forest trees, which bear their fruit chiefly on their upper branches, was another serious impediment to the proper progress of this research.

I adduce these particulars merely to explain the great length of time that has elapsed since the task was undertaken, and as some apology for the fragmentary nature of this first instalment of my results.

* M. Treub, "Sur les Casuarinées et leur place dans le Système Naturel," *Ann. du Jard. Bot. de Buitenzorg*, x. pp. 145-231.

Examination of the fresh material was, as I have said, made as far as possible when it was gathered, and, although the exceeding delicacy of manipulation required of an embryologist, if trustworthy results are to be obtained, could scarcely be called into play when material was accumulating so fast, I soon found myself in contact with new and interesting forms which I had never met with before. It seemed advisable that I should ascertain the whole course of development of the embryo-sac and its contents in at least one particular group of genera and thus be sure of my ground in one connexion. I chose the Cupuliferæ, which, although more normal in many respects than some other groups, will prove, I think, on further description, to be an interesting point of reference when we pass to others.

CUPULIFERÆ.

This group is referred to in the 'Genera Plantarum' of Bentham and Hooker as the Quercineæ, under which name are placed the four genera *Quercus*, *Castanopsis*, *Castanea*, and *Fagus*. For embryological purposes it appears to me best to give some account of *Fagus* first.

FAGUS. (Pl. LXVII. figs. 1-11; Pl. LXVIII. figs. 12, 13.)

The species selected for observation was *Fagus sylvatica*, Linn., and to this I refer by its generic name simply. Early in April we can just distinguish the buds which contain the young flower-heads from the less bulky leaf-buds. If the young female flowers are now examined we find the ovules are already laid down, and rudiments of the inner integument are seen to be forming, partly at the expense of the epidermis. Simultaneously one or more contiguous epidermal cells at the apex of the young nucellus divide by periclinal walls. While this is taking place there is differentiated immediately below them a layer of subepidermal cells, generally from three to six in number, as seen in a longitudinal section, which forthwith begin to divide by parallel straight walls in the same manner as the apical epidermal cells, *i. e.* periclinally (figs. 1, 2).

Although nearly the whole of the nucellus is constructed from these two layers, only the central strands maintain the regular mode of division and become strongly marked out from the peripheral tissue. In a transverse section this core of the nucellus generally separates from the surrounding tissue and leaves a ring of nucellar tissue within the double ring of the transversely-cut integuments. As development proceeds the epidermal cap spreads over the whole upper surface of the nucellus and grows to be about thirteen cells deep, thus forming nearly a third of its bulk (fig. 9, *e.p.*).

The central strands of the nucellus, as follows from what has been said, extend down to the level of the insertion of the inner integument, and are composed of large prismatic cells—often hexagonal in transverse section, and, especially in the central regions of the nucellus, elongated in the direction of its axis. It not unfrequently happens that in one strand certain partition-walls are delayed in development, and thus we get one long cell corresponding to a tier of three or more cells. These long cells are often crowded out (fig. 10, *l.c.*). It seems, however, that, at least occasionally, the embryo-sac may arise in this manner (fig. 8, *l.c.*). This central series of coaxial strands appears to be "sporogenous tissue," although it is very rare in *Fagus* to find more than one cell continuing its

development, though such a case is shown in fig. 9, *e.s.*, *e.s.* I have not been able to trace the organic base of these axial strands in material gathered later than May 14, ten days after the date of the ovule drawn in fig. 3. In the case referred to, the tendency to develop one strand more prominently than the rest, which we sometimes observe even in *Fagus*, is shown by the semi-diagrammatic figure (fig. 5), which should, however, be contrasted with that given in fig. 6.

The embryo-sac most frequently develops from a cell that lies about fourth or fifth down from the epidermal cap. There is no other mark by which to distinguish it from its sister cells above and below it in an axile row than the formation of the two nuclei which take up their position at either end of the cell (fig. 7, *e.s.*). But we may, perhaps, from analogy with other Angiosperms, tentatively regard the cells intervening between it and the epidermis as tapetal.

Pollination occurred on May 9, just three weeks before the pollen-tubes reached the embryo-sac. On May 24 the ovarian cavity was crowded with branching pollen-tubes, three or four of which have been seen to enter a micropyle simultaneously (fig. 11). They enter the epidermal cap and make their way to the apex of the embryo-sac. Here one was observed to penetrate for a short distance between the two synergidæ which abutted on the apex of the sac, but I have not succeeded in observing the transfer or even presence of the male nucleus. The converging tiers of cells of the epidermal cap are doubtless of service in directing the course of the pollen-tube, and their marked turgidity is probably also advantageous, for the cells collapse and flatten after fertilization has taken place. Fig. 12 shows a pollen-tube plunging between these receptive cells.

The embryo-sac presents nothing abnormal up to the period of fertilization. At this time the antipodals acquire a thick cellulose wall, and are thus prevented from entering the cæcum, which is now extruded from the base of the sac. The definitive nucleus enters this cæcum, which sometimes reaches to the base of the nucellus, taking its course down one of the tiers of cells already described (fig. 13).

To sum up:—*Fagus* is normal in the structure of its embryo-sac and in the course taken by the pollen-tube. Its sporogenous tissue agrees far more with Strasburger's well-known description of *Rosa livida* than with Treub's account of *Casuarina*, and the cæcum is only formed on, or shortly before, fertilization.

CASTANEA. (Pl. LXVIII. figs. 14–21; Pl. LXIX. figs. 22–27.)

The species examined has been *Castanea vulgaris*, Lam. (*C. sativa*, Mill.), and to this I refer when employing the generic name. The appearance of the young nucellus is best understood by a reference to fig. 14. Essentially the same structure obtains as in *Fagus*, but the lateral strands no longer, in their early stages, suggest a sporogenous tissue. The central strand of five to eight cells is obviously the archesporium. The two to four upper square cells, the uppermost of which abuts immediately on the epidermis, appear to be very evidently tapetal. The embryo-sac lies immediately below them, and we find two sister cells continuing the row, which I have several times observed to be bi-nucleate (fig. 18, *b.c.*). Occasionally these cells undergo further division, as is shown in fig. 20, *ax.*

They enlarge and compress the neighbouring cells, some of which may be observed to

resist the pressure and become spindle-shaped, and occasionally bi-nucleate, themselves, but it is difficult to discriminate between a nucleus breaking down in process of degeneration and an active one when the cells are surrounded by a degenerating tissue. It is, however, important to bear in mind that small spindle-shaped cells appear around the base of the axial strand, and are formed sometimes by divisions arising obliquely in this row, and apparently sometimes by secondary modification of members of the immediately contiguous lateral strands. They are generally at first smaller than their neighbours, although some are destined later to undergo a strikingly new development (figs. 16, 17).

Meanwhile the cell about to become the embryo-sac has enlarged. Its nucleus has divided into two and then into four in a manner entirely normal. Later on the polar nuclei fuse and form an exceptionally large and conspicuous definitive nucleus (fig. 21, *d.n.*) which is connected with other contents of the sac in all directions by anastomosing moniliform strands, often of great tenuity. As previously described, in *Fagus* this nucleus passes into the cæcum, which is now extruded. In *Castanea* the cæcum breaks its way through the side-wall of the nucellus near its apex, and runs down between the long cylindrical nucellus and the inner integument (fig. 20) and very quickly reaches the base of the nucellus. The antipodals are consequently not thrust to one side, and they arrange themselves in a tier as shown in fig. 21, *a.* It is around this pointed organic base of the embryo-sac that we first observe the appearance of small tracheïdes, one or more of which ultimately reach a considerable size and become remarkably conspicuous objects under the microscope. As these at once suggest an homology with the tracheïdes in the sporogenous tissue of *Casuarina* as figured by Treub* in plates xix. and xx., I have given several drawings of them (figs. 21–27). It is by far the commonest result in a longitudinal section to find but one tracheïde; indeed fig. 22 represents the only preparation I have obtained that shows a complete and symmetrical arrangement of such tracheïdes around the antipodal extremity of the embryo-sac. Their function is as unaccountable to me as that of the tracheïdes in *Casuarina* was to Treub; but I do not think we can doubt their homology when we bear in mind the undoubtedly sporogenous nature of the tissue surrounding the embryo-sac in *Fagus*, and the great structural resemblance of the nucellus of *Castanea* to that of *Fagus*. Moreover, they are never found *outside* the well-marked degeneration-area which extends for a short distance down the centre of the nucellus, and they appear to carry on their development even when the embryo-sac, as in many of the abortive ovules, has ceased to expand. The whole character of these tracheïdes is so distinct from that of any other cells in the nucellus, whether of *Fagus*, *Quercus*, or *Castanea*, that I have little hesitation in regarding them as a vestige of some long-lost structure. But the strongest evidence in support of this claim lies in a comparison of the spindle-shaped cells, and their later stages as tracheïdes, with the long, narrow, pointed cells about to be described in the case of *Carpinus* (Pl. LXXI. fig. 48, *s.*). We see there the coaxial system of strands is almost completely merged in one part of its course into a mass of these long pointed cells. The only suggestion of these that we find in *Fagus* is the tendency of the walls

* Ann. du Jard. Bot. de Buitenzorg, x.

in the lower regions of the strands to be more or less oblique instead of exactly transverse—as are the walls which give the characteristic appearance to the upper part.

The *Castanea* material I have worked upon was gathered in the East of England and gave very unsatisfactory results in the later stages. I hope this year to obtain material from a district in which this species is better able to carry on the development of its fruit. Judging, however, from flowers gathered from August 6, when I first find the tracheïdes appearing, until August 22, I should be inclined to say they are not constantly present. A complete series of sections of an ovule cut with the microtome often reveals no trace of a tracheïde, and this inconstant appearance supports the view that they are vestiges of some former structure.

QUERCUS. (Pl. LXIX. figs. 28, 29.)

This genus has not afforded me anything distinctive to relate. It closely resembles *Castanea* in the structure of the nucellus, although this never attains the great length that is so characteristic in *Castanea*. The axial strand, the position of the embryo-sac in this, all the processes in the embryo-sac—including the extrusion of the cæcum (fig. 29, *cæ.*)—and the arrangement of the antipodals in a pointed base, can be readily demonstrated to be similar. I have been able to verify Hofmeister's observations on the course of the pollen-tubes, several of which enter and descend a single micropyle. The pollen-tubes form short blind branches as shown in fig. 28, *c., c.* The embryo, being attached to the apex of the extruded embryo-sac, is to be seen lying almost in the micropyle up which the embryo-sac pressed before fertilization.

CASTANOPSIS.

This genus still awaits investigation, but I hope soon to have the material in hand. The fruit takes two years to develop, as is well known to be the case with *Q. rubra*, *Q. Cerris*, and many other species of *Quercus*.

On leaving the Cupuliferæ we enter upon the examination of two groups of the Amentiferæ which, so far as yet investigated, reveal a striking phenomenon in the abnormal course of the pollen-tube, which does not enter the micropyle, but penetrates the nucellus from the chalazal region. This exceedingly interesting fact was first noted by Treub for the case of *Casuarina*. I hope now to show that it also occurs in *Betula* and *Alnus*, *Corylus* and *Carpinus*, and I will refer to this group of genera by the distinguishing name of "Chalazogamic Amentiferæ." The whole account of the course taken by the pollen-tube, and other associated topics, will be found further on, but I will first give a short description of the embryology of representative species of each genus.

BETULA ALBA, Linn. (Pl. LXIX. figs. 30, 31.)

The structure of the young nucellus can be seen from the longitudinal section represented in fig. 30. We see again the coaxial system, the archesporium abutting on the epidermis, and this epidermis two-layered as in an early stage of *Fagus*. It is the more

interesting to note this resemblance, as in a short paper published March 1893, but which I unfortunately overlooked until the middle of May, the writer expressly states, "In the nucellus there is no axial row of cells, which usually appears distinctly in the rest of the Angiosperms" *.

The central axis gives rise eventually to the embryo-sac, which is solitary and at no time in its development forms a cæcum. The contents of the sac are normal, and the egg-cell is ready for fertilization at the end of May. In the material gathered in the season of 1892, pollination took place during the first week of May and fertilization during the first week of June. The nucellus never attains to anything but a very inconsiderable size, as the whole fruit is adapted for wind-dispersal. The ovules given in longitudinal section (figs. 30 and 31) lay at right angles to the axis of the fruit, but before an ovule attains its full development it becomes anatropous, and the stylar canal is closed by the outgrowth of loose cellular tissue. These organogenetic details being common to the other chalazogamic Amentiferæ, and deriving their interest for the embryologist chiefly from their bearing on the course of the pollen-tube, I will enter into them more fully when treating of that subject.

ALNUS GLUTINOSA, Medic. (Pl. LXIX. figs. 32, 33; Pl. LXX. figs. 34-38.)

The coaxial system of strands still obtains in the nucellus of *Alnus*. The tapetum above the solitary embryo-sac is proportionately longer than in *Betula* and in the Corylaceæ. The embryo-sac lies very deep down in the nucellus, and the tapetum, down which for a considerable part of its course the pollen-tube travels, has a grumous aspect. It is not, perhaps, an improbable conjecture that the cells exert a chemical stimulus upon the tube which enables it to arrive at the apex of the sac after a very sinuous course. The contents of the embryo-sac are normal (fig. 33), and the antipodals resemble those figured by Treub for *Myrica* rather than those of *Castanea*, although there is no essential difference, and in all cases cellulose walls are conspicuously present. I have given a figure of the embryo of *Alnus*, which is again normal (fig. 37).

CORYLACEÆ.

In this group we meet with a far more strongly-developed sporogenous tissue than in *Betula* and *Alnus*, although it much resembles theirs in character.

In *Corylus* and *Carpinus* we find for the first time among the British Amentiferæ a large number of macrospores which continue their development up to the stage of forming two or even four nuclei. In those which continue their development the characteristic contents of the normal angiospermic embryo-sac are present, but the synergidæ and antipodals are difficult to recognize, except when first formed. The egg-cell is always a prominent object in the upper region of the sac, whilst the definitive nucleus, in a well-developed cæcum of the sac of *Carpinus*, attains a large size, reminding one of those of *Castanea*.

* "Zur Embryobildung der Birke," by S. Nawaschin, in Bull. Acad. Imp. Sci. St. Pétersb. sér. iii. xxxv. no. 3. In this paper Mr. Nawaschin records his discovery of the true course of the pollen-tube in *Betula*.

CORYLUS AVELLANA, Linn. (Pl. LXX. figs. 39-43 ; Pl. LXXI. figs. 44-47.)

In this genus we find much that is new and of interest to the embryologist. Although the sporogenous tissue is based on the coaxial system, and the strands are particularly clearly marked out in the tapetal region, there is less regularity in the region immediately above the pedicel. The cells are there smaller and more granular, and several early show signs of further development. The sections given in figs. 41, 42, 43 were prepared in June and July 1892 from the fresh material. In fig. 42 one embryo-sac had separated from the section, and its position is indicated only by a gap in the nucellar tissue (*p.e.s.*). Later on, one of these embryo-sacs sends out a protuberance which passes down towards the base of the nucellus. Figs. 46 and 47 represent a cæcum with a pollen-tube abutting upon it. Figs. 44 and 45 also represent a pollen-tube—*in situ*—passing up the nucellus from the chalaza. The cæcum attains considerable length only when the embryo begins to develop. In this, *Corylus* offers a striking contrast to *Carpinus*.

On two occasions I have found two fully-developed embryo-sacs, and in these cases they elongated side by side. The egg-cell covers itself with a cellulose wall and becomes attached to the apical part of the wall of the sac. The synergidæ never become so large and conspicuous as those of the Cupuliferæ.

CARPINUS BETULUS, Linn. (Pl. LXXI. figs. 48-50 ; Pl. LXXII. figs. 51-60.)

There is much that is similar to *Corylus* in the early stages of *Carpinus*. Fig. 48 shows the area of the sporogenous tissue and pedicel as marked out in a longitudinal section. The embryo-sacs arise chiefly from the central strand, and two or more take on a full development and send long cæca down to the base of the nucellus. In fig. 49 only one of these is indicated, fig. 54 shows two, and fig. 53 four ; but it is rare to find the cæca quite in the same plane. These cæca are best seen in a comparatively thick section of the nucellus, which is composed of remarkably large and transparent cells in *Carpinus*. Such a section sometimes reveals a nucellus riddled by these tubes, which necessarily come into close contact at the narrow base of the nucellus. These tubes reach their full length before any endospermic nuclei are formed, and the definitive nucleus is conspicuous in the cordon of protoplasm which reaches from the apex to the base of the embryo-sac. It is by means of these dense continuous tongues of protoplasm that we can best trace the course of the cæca in *Carpinus*, and it is along these that I have on several occasions traced a pollen-tube, which, after entering the base of the cæcum, makes its way to the egg-cell. Fig. 50 shows one still persisting after fertilization has occurred.

The Pollen-tube.

This subject may be advantageously treated under the three headings of

- Resting Stage,*
- Chalazal Route,*
- Branching of the Tube.*

Resting Stage.—In Goebel's 'Outlines of Classification and Special Morphology,' I

find on p. 392 of the English edition the following statement, which, I think, is calculated to give an erroneous impression respecting the process of fertilization in *Quercus* and *Fagus*:—"Fertilization is usually accomplished in a very short time after the pollen-tube reaches the apex of the embryo-sac, yet the cases are not few in which a long time elapses between the arrival of the pollen-tube and the commencement of the development which it excites—several days or weeks in woody plants, as *Quercus*, *Fagus*, &c.; almost a year in the American oaks, which take two years to ripen their seed."

It is here implied that the pollen-tube does not enter on its resting stage until it reaches the embryo-sac, whereas the organogenetic researches of Baillon show that in *Quercus* no vestige of the ovule is present at the time of pollination, and I have failed to demonstrate the presence of tubes in the styler canal or ovarian cavity until the period of fertilization approaches. The tubes remain latent during the four months that the ovary is developing in the case of such a species as *Quercus Robur*, which ripens its fruit in one year; but for so long a period as eleven months in other species of *Quercus*, which take two years to ripen their fruit. The case is very similar in the Betulineæ and Corylaceæ.

In the material which I have examined gathered in 1892, the pollen-tubes remained in a resting stage in the case of *Corylus* from February 5 until June 21, *i. e.* over four calendar months; in the case of *Carpinus*, from May 6 to July 1, nearly two months; in the case of *Alnus*, from March 23 to June 17, nearly three months; and in the case of *Betula*, from May 6 until June 8, one month.

The case of *Fagus* is particularly easy to investigate. The ovule represented in Pl. LXVII. fig. 4 represents the stage reached when pollination occurred on May 9, but no pollen-tubes could be found in the cavity of the ovary until May 21, when they were observed descending from the styler canal in great numbers, and the rapidity of their growth was also curiously manifested by a chemical reaction that occurred in material gathered and pickled during the week beginning May 21. Large crystals of calcium oxalate were deposited on the interior wall of the ovary around the ovules, and often even on the free pollen-tubes as they left the styler canal and entered the larger cavity of the ovary. But even on May 24 no pollen-tube had reached the nucellus. Three days were required by them to find their way down the very long micropyle. The tunnelling of the epidermal cap was accomplished only on the last two or three days of May. The embryo begins to elongate almost immediately on the pollen-tube reaching the embryo-sac, examples having been found of the various stages in material gathered on the same day.

In order to investigate whether anything unusual occurred in the young pollen-tube which would render it specially fitted for so long a resting stage, I made several cultivations of the pollen-grains of *Carpinus* and found that they germinated very freely, thrusting out the intine at one of the five specially-prepared spots.

For two days elongation occurred, and then a thickening of the apical region took place, and this distal part of the tube became enlarged (Pl. LXXI. figs. 46 and 47). Into this there passed two nuclei, and the tube not only became plugged at its point of junction with the grain, but also I observed it once or twice to be again plugged immediately above the enlarged tip, which thus became virtually an isolated spore like a pollen-grain, which,

though it had lost its outer coat, would in the normal state of things be safely housed or encysted in the tissue of the style.

After observing this result of germination of pollen-grains, I was in some doubt as to how far it might be merely a pathological arrest of growth. I treated a number of fairly thick longitudinal median sections of the ovary of *Carpinus* with H_2SO_4 in the hope that maceration of the surrounding tissue would bring into view any trace of the pollen-tubes in the stylar cylinder. Figs. 59 and 60 (Pl. LXXII.) represent one of these macerated sections, taken from material gathered July 1st, 1892; but I have as yet failed to obtain further evidence for the existence of a secondary pollen-grain or portion of tube encysted in the tissue of the style.

Chalazal Route.—Leaving now the subject of the resting stage undergone by the pollen-tube, let us turn to a phenomenon which is less universal in Amentiferæ, and, so far as is at present known, is peculiar to the three groups Casuarinæ, Betulaceæ, and Corylaceæ. I refer to the route taken by the pollen-tube to gain access to the embryo-sac.

Schacht, writing just forty years ago, claims to have observed a portion of the pollen-tube in the nucellus of *Corylus*. He writes: "Several times have I seen in successful longitudinal sections a tubular cell in the tissue of the nucellus, which was united with a racemose body of cells lying in the apex of the embryo-sac. The tubular cell, *cut off above*, is the penetrated pollen-tube" *.

Here Schacht had probably made a very similar observation to that recorded by Hofmeister † in 1858 when he said, respecting *Betula* and *Carpinus*, that the cell-layers covering the apex of the sac are tunnelled by the pollen-tube. This statement has been a fruitful source of error.

Treub quotes the passage as sufficient evidence for the normal course being pursued by the pollen-tube in these trees, and did not consider it necessary to investigate the matter further, although, as it appears, such a step would have led him to conclusions wholly different from those he drew from the literature on the subject.

The pollen-tube does indeed in some instances (e.g. *Alnus*) tunnel the cell-layers which cover the apex of the embryo-sac, but it has, in such cases, reached these cell-layers by a route wholly as abnormal as Treub has pointed out in the case of *Casuarina*. It descends from the tissue at the base of the stylar rudiments and, running parallel with the vascular bundle of the raphe, turns abruptly up into the nucellus on reaching its chalazal region (Pl. LXX. fig. 38). The base of the nucellus will be observed to lie in such a direction (figs. 32 and 34) that the pollen-tube following the trend of the cells in the neighbourhood of the vascular bundle of the raphe cannot fail to find its way into the nucellus. This position of the base is gradually acquired by unequal growth of the

* 'Botanische Zeitung,' 1854: Schacht, "Beiträge zur Anatomie" &c. The "racemose body of cells" of the quotation refers probably to a group of three or four embryo-sacs which lie near the apex of the nucellus.

The "tubular cell cut off above" I think very likely not a pollen-tube, but one of the long tapetal cells so frequently found in this region, and often strikingly suggestive of bits of old pollen-tubes. On the whole I cannot accept Schacht's statement as evidence for the presence of a pollen-tube in this position.

† Pringsheim's 'Jahrbuch,' 1858: Hofmeister, "Embryobildung der Phanerogamen."

two halves of the ovule which cause the very characteristic curvature of the ovules of *Alnus*, *Betula*, *Corylus*, and *Carpinus*. The position is strongly contrasted with that of *Fagus* and *Quercus*, although these ovules are also anatropous; but, at the same time, resembles what, from Treub's figures, we observe to be the case in *Casuarina* (plates xxii. and xxiv.)*.

From the difference in the one case and the agreement in the other we are, I think, entitled to regard this feature as a special adaptation for chalazogamy.

I have given two figures of pollen-tubes in contact with the base of the embryo-sac cæca (*Corylus* and *Carpinus*). In the case illustrated by figs. 46 and 47 (Pl. LXXI.) we find the tube abutting on the comparatively short cæcum of *Corylus*. In figs. 44 and 45, which also exhibit the tube ascending the nucellus, the lower part of the tube alone is represented.

In figs. 55 and 56 (Pl. LXXII.), which illustrate a similar case in *Carpinus*, contact has taken place quite at the base of the nucellus. These instances, and especially the perforation of the wall of the embryo-sac at the spot abutted upon by the pollen-tube, as shown in fig. 55, prepare us for the next stage, which we see delineated in figs. 50 and 51. The pollen-tube enters the base of the fertile embryo-sac in *Corylus* and *Carpinus*, and, continuing its course up the whole length of the cæcum, eventually reaches and fertilizes the oosphere by direct contact. Whether this has already surrounded itself with a cell-wall or not I have not conclusively determined, although many of the preparations so far made suggest this to be the case, as I have repeatedly found a unicellular egg-cell clothed with a cell-wall where I have failed to demonstrate any vestige of a pollen-tube; and yet fig. 50 proves it capable of persisting until the oospore has twice divided. The inconspicuous development of the synergidæ and their early dissolution are now explained. They are not required to assist in any way the act of fertilization, for the pollen-tube reaches the oosphere from below, having previously entered the embryo-sac in its basal region.

Fig. 32 taken with fig. 34 (Pl. LXX.) has enabled me to construct the course taken by the tube in the nucellus of *Alnus* as given in the diagrammatic drawing, fig. 38. From the chalaza it runs up more or less by the tiers of large cells which constitute the stalk-like prolongation or pedicel (Treub) of the sporogenous tissue already described. It leaves this, and ascends to a level considerably above the apex of the embryo-sac, and then, turning down again, presents the appearance of a tube pursuing a normal course. It was doubtless this last part of the course that was observed by Hofmeister.

Branching.—The pollen-tube presents another point of likeness with that of *Casuarina*, inasmuch as it sends out a short recurved branch on entering the nucellus (fig. 36, b.).

This branching of the pollen-tube is a very general feature in the Amentiferae. Even in those genera in which we do not find the chalazal course of the tube, we find a process of bifurcation still very widely present. Schacht, in the above-mentioned paper, gives drawings of the pollen-tubes of *Fagus*. I include a figure of the tubes of *Quercus* (Pl. LXIX. fig. 28). The character of the branching is, in *Fagus*, the simple bifurcation of the tube into what appear to be equivalent tubes, but in the case of the chalazogamic

* Treub, *loc. cit.*

Amentiferæ the recurved branches found are simply cæca and probably contain no portion of the fertilizing element.

There are several cases of branching pollen-tubes recorded by Hofmeister among the Monocotyledons in the 'Neue Beiträge zur Kenntniss der Embryobildung der Phanerogamen,' 1861. The branched tubes of *Pothos longifolia* are represented in Taf. x. figs. 1 and 2, and those of *Hippeastrum aulicum* in Taf. xiii. figs. 4 and 6. But more interesting examples of the branching of pollen-tubes occur among the Gymnosperms, where we find in the Cupressineæ one tube applying its broad extremity to the necks of a whole group of archegonia at the same time, and then forming short narrow protuberances which grow down into the separate archegonia.

Comparative account of the Archesporium in the foregoing Genera.

We find in reviewing the various examples described in the preceding pages certain features in their archesporium which they have in common, not only with one another, but with the normal type which has been shown by Strasburger and others to be almost universal among the Angiosperms.

Thus we observe in all eight genera the subepidermal origin of the archesporium and also the presence of tapetal cells. Again, the archesporial cells are arranged in strands, formed at first of definite tiers of cells cut off from their respective initial subepidermal cells. But whereas in the normal Angiospermic type we find but one of these strands, which is therefore named "the axile row," in the genera now under consideration we find a large number of these rows derived from a subepidermal layer, and it is difficult in many cases to point out one row as more distinctly characterized than some others (Pl. LXVII. fig. 8, Pl. LXXI. fig. 48). This system of strands, forming what I have previously referred to as a *sporogenous tissue*, is connected with the base of the nucellus by a regular series of cells which I have referred to as the *pedicel* of the sporogenous tissue.

Again, we find oblique divisions occurring in the cells of this coaxial system of strands which lie between the tapetum and the pedicel of the sporogenous tissue. The spindle-shaped cells thus arising, in the case of *Castanea*, develop into large and conspicuous tracheïdes, but it is chiefly in the Corylaceæ that they become a marked feature, though they do not form tracheïdes. Without trespassing too far into the region of theory, it may be of interest to note that the sporogenous tissue of *Fagus* seems to belong to a more primitive type than we find in the other genera. This view has independent support from Eichler's opinion, who deduced from the floral morphology of the Order that the Cupuliferæ are a less specialized group than the Corylaceæ and Betulineæ. In *Fagus* also there are two integuments to the ovule and a very well-developed epidermal cap. In the latter character, among others, there is considerable agreement between the nucellus of *Fagus* and those of *Gnetum*, *Taxus*, and other Conifers. From the *Fagus* type we find, on the one hand, a reduction in the extent of the archesporium in the case of the other Cupuliferæ, until there is very little that is not normal in *Quercus*; and on the other hand, a reduction in bulk in the case of Corylaceæ, Betulineæ, and Juglandaceæ, although in Corylaceæ, especially, we can still claim for a large part of the tissue of the nucellus a sporogenous nature.

Relations of the Amentiferæ to Casuarina as revealed by Embryology.

This part of my subject cannot be dealt with exhaustively until further and deeper researches have been made. The agreement I have to point out is so mixed up with striking distinctions that we cannot, so far as our knowledge at present extends, in any way claim to have found a counterpart in our British Amentiferæ to the extraordinary facts recorded by Treub for *Casuarina*. We know nothing of an embryo-sac without antipodals, for they are present and very conspicuous in the British Amentiferæ. In *Betula*, *Alnus*, and the Cupuliferæ I have observed the fusing of the polar nuclei, and in every genus the presence of synergidæ and *naked* egg-cell. But Treub concludes his paper with the words:—"A moins que de nouvelles recherches ne viennent nous apprendre le contraire, il faut considérer la famille des Casuarinées comme la seule de la classe des Chalazogames." These new researches have now been made, and it will be no longer possible to classify the Casuarinæ by themselves as Chalazogams. *Alnus*, *Betula*, *Corylus*, and *Carpinus* are also Chalazogams, and if we except the distinctions I have just pointed out in the early stages of the embryo-sac, almost all we have to record are, more or less, points of agreement. It will be convenient here to summarize the points of agreement between *Casuarina* and the British Amentiferæ:—

1. In the case of the Corylaceæ and Betulineæ there is the prominent fact of their chalazogamy and all its concomitant adaptations. Among these I may mention the fusion of the tissue of the styler region with the central strand, which is itself formed by the fusion of the two parietal placentæ, which have grown centripetally until they met and formed one strand, from the upper portion of which the now anatropous ovules spring (cp. figs. 39 and 40, also figs. 34 and 35, Pl. LXX.).

Another special adaptation is the projection of the base of the nucellus into such a position that the pollen-tube, after travelling down the raphe in a course parallel with that of the vascular bundle, and following the trend of the cell-rows in the chalazal region, inevitably enters through the circular base of the nucellus (figs. 32, 34). We also see the sheath of crystals, which is so prominent a feature in *Casuarina*, although it would appear that our British Amentiferæ have not specialized it to assist in the act of fertilization, as has occurred in *Casuarina* (figs. 34 and 35, also fig. 39).

2. The sporogenous tissue, already sufficiently described in another paragraph, although presenting striking points of difference in detail from that of *Casuarina*, constitutes, in the main, an important point of agreement.

3. The branching of the pollen-tube, especially the formation of recurved cæca before its entry into the nucellus in the chalazogamic genera, and the resting stage that it passes through in the styler tissue may, perhaps, be mentioned here.

4. The prevalence of cæca formed by the embryo-sac is a remarkable feature of agreement. This character is so widely represented in the British Amentiferæ that it may fairly be regarded as of taxonomic value. The fact that the tails of the sterile macrospores in *Casuarina* serve for the unimpeded pathway of the pollen-tube up the nucellus, while that of *Fagus* is simply foraging for the needs of its embryo, only offers another example of the special adaptation to new functions of an organ already acquired by

inheritance. Whether the cæca were originally acquired to assist the embryo-sac to come in contact with the pollen-tube or for foraging purposes is immaterial from the present point of view. Physiological considerations in no way detract from the classificatory value of these cæca.

5. The presence of tracheïdes around the base of the embryo-sac in *Castanea* is a curious and unexpected link between the Cupuliferæ and *Casuarina*. Alone this feature might be regarded as too detached and fragmentary to be of any value as a point of agreement, but when we remember that we can suggest no function subserved by them either in *Casuarina* or in *Castanea*, and that their appearance is inconstant in both cases, the probability is increased that they represent some former organ* possessed by the common ancestor of *Casuarina* and the Cupuliferæ. It might be noted here that although the ovules of our British chalazogamic Amentiferæ possess but one integument, *Casuarina*, in common with the Cupuliferæ, possesses a second.

Conclusion.—There is still a large part of my work to be done. I have not yet relinquished all hope that the Amentiferæ are a field of research in which we may find some clue to the solution of the vexed problem of the homology of the antipodals and of the fusion of the polar nuclei of the embryo-sac, and it will be partly with this object in view that I continue and extend my investigations.

DESCRIPTION OF THE PLATES.

PLATE LXVII.

Fagus.

- Fig. 1. A longitudinal median section of young ovule of date May 4, 1892. *ep.*, the beginning of the apical divisions of the epidermis above the archesporium; *a.*, archesporium mother-cells; *i.*, integument forming partly at the expense of the epidermis.
- Fig. 2. A similar section to the above. This figure shows, when contrasted with the previous, the early laying-down of the anatrophy of the ovule. The funicle of fig. 1 lies to the right of the figure, that of fig. 2 to the left.
- Fig. 3. The same a little later. *ep.*, the beginning of the epidermal cap; *a.c.*, the lowermost cell of the most strongly differentiated axial strand. The two nucleoli are very frequently found in central cells at this stage.
- Fig. 4. The same at a still later date, May 10, 1892. The epidermal cap is here five cells deep.
- Fig. 5. A median longitudinal section of nucellus of date May 14. The central strand was more clearly marked out than the lateral ones and showed a pointed base. (Semi-diagrammatic.)
- Fig. 6. Ditto, but lateral strands appear of equivalent value. (Semi-diagrammatic.)
- Fig. 7. Ditto, gathered May 21. *ep.*, epidermal cap now ten cells deep; *es.*, first division of nucleus of embryo-sac.
- Fig. 8. Ditto, gathered May 18. *ep.*, as before; *t.*, tapetum; *l.c.*, long cell equivalent to three of the

* Possibly a vascular strand connecting the chalaza with the sporogenous tissue.

contiguous tier—apparently marked out as an embryo-sac; *v.*, vacuole. The strands can be seen here to continue to the base of the nucellus although the section is not exactly median throughout.

- Fig. 9. Ditto, gathered May 28. Shows development of embryo-sacs in *two* contiguous strands. The more advanced one (above) is nearly ready for fertilization. The antipodals have become detached by the razor from the upper part, which contains still the synergidæ and polar nuclei. *ep.*, epidermal cap now thirteen cells deep; *t.*, tapetum; *e.s.*, *e.s.*, embryo-sacs; *a.*, antipodals; *f.*, polar nuclei; *p.*, pedicel.
- Fig. 10. Portion of the sporogenous tissue of nucellus as it appears in longitudinal section. *l.c.*, long cell being crowded out.
- Fig. 11. This drawing is taken from a thick section cut in the fresh material. It shows a superficial view of apical region of epidermal cap, the cells of which are turgid; between these plunge three pollen-tubes (*p.t.*). *m.*, micropyle; *r.ep.*, receptive cell; *n.*, nucellus; *int.*, integument.

PLATE LXVIII.

Fagus.

- Fig. 12. Longitudinal section of upper part of nucellus showing pollen-tube (*p.t.*) tunnelling epidermis (*ep.*). *s.*, synergidæ; *e.c.*, naked egg-cell.
- Fig. 13. Portion of longitudinal section of nucellus showing embryo-sac with extruded cæcum. *a.*, antipodals; *d.n.*, definitive nucleus; *b.c.*, base of cæcum; *s.*, strand down which it has made its way; *e.s.*, main portion of embryo-sac.

Castanea.

- Fig. 14. Longitudinal median section of nucellus, dated July 8, 1892. *a.r.*, axile row already becoming marked out from contiguous strands.
- Fig. 15. Ditto, eleven days later. *t.*, tapetal cell; *m.s.*, mother-cell of embryo-sac.
- Fig. 16. Ditto, July 25. *t.*, tapetal cell; *s.*, pointed cell.
- Fig. 17. This section belongs to an ovule which had ceased to develop normally. The tapetal cells (*t.*) had degenerated and the appearance of the contents of the embryo-sac (*e.s.*) is not normal. *b.c.*, basal cells of axile row; *b.s.*, spindle-shaped cell.
- Fig. 18. Longitudinal section showing base of axile row, also from an aborting ovule. *tr.*, mother-cell of tracheïde; *b.c.*, bi-nucleate cells of axile row below the embryo-sac.
- Fig. 19. Upper part of axile row. *m.*, micropyle; *e.i.*, epidermis of inner integument; *t.*, tapetum; *n.e.c.*, naked egg-cell; *a.*, antipodals.
- Fig. 20. Longitudinal section of upper part of nucellus, August 22. *m.*, micropyle; *i.i.*, inner integument; *e.s.*, embryo-sac; *d.n.*, definitive nucleus; *m.s.*, moniliform strands of protoplasm; *cæ.*, cæcum of embryo-sac; *ax.*, basal part of axile row; *s.c.*, pointed cells.
- Fig. 21. Ditto. *d.n.*, definitive nucleus; *a.*, antipodals; *tr.*, tracheïde; *b.c.*, basal cell of axile row.

PLATE LXIX.

Castanea.

- Figs. 22–27. Longitudinal sections of nucellus to show tracheïdes (*tr.*). *a.*, antipodal; *d.*, degenerating area; *m.*, micropyle; *e.s.*, embryo-sac; *d.n.*, definitive nucleus, faintly indicated only, as the section had been treated with Eau de Javelle.

Quercus.

- Fig. 28. Pollen-tubes as found growing freely in the ovarian cavity and entering micropyle (*m.*). *o.i.*, outer integument; *c.*, cæca of pollen-tube.

Fig. 29. Longitudinal section of nucellus and part of inner integument of *Quercus rex*. *m.*, micropyle; *e.s.*, embryo-sac; *a.*, antipodals; *b.c.*, basal part of axile row; *nu.*, nucellus; *cæ.*, cæcum of embryo-sac; *i.i.*, inner integument.

Betula alba.

Fig. 30. Longitudinal section of nucellus, May 16, 1892. *a.c.c.*, archesporial central-cell dividing.

Fig. 31. Ditto a little more advanced. *a.*, archesporium.

Alnus glutinosa.

Fig. 32. Outline of upper part of long. sect. of ovary and one ovule just fertilized. *p.t.*, pollen-tube; *e.s.*, embryo-sac; *c.a.*, central axis; *lo.*, loculus.

Fig. 33. Embryo-sac of fig. 32 enlarged. *p.t.*, pollen-tube; *s.*, synergidæ; *e.c.*, egg-cell; *f.*, fusing polar nuclei; *a.*, antipodals.

PLATE LXX.

Alnus glutinosa.

Fig. 34. As in fig. 32, but here is shown the pollen-tube entering the chalazal region, also a transverse section of the tube to the right above the level of embryo-sac. *s.*, stylar rudiments; *c.*, cellular tissue; *ch.*, sheath of crystals; *p.t.*, pollen-tube; *e.s.*, embryo-sac.

Fig. 35. Transverse section of ovary taken at level of dotted line *c.c.* in fig. 34. *p.*, loose parenchyma showing no stylar canal; *ch.*, crystal sheath and lignified tissue.

Fig. 36. Enlarged drawing of base of ovule of fig. 34. *ch.*, chalazal region; *p.t.*, pollen-tube; *i.r.*, integument (level of insertion of) on the side nearer the raphe; *i.d.*, do. on the distal side; *b.*, branch of pollen-tube.

Fig. 37. Embryo of *Alnus*, July 25, 1892. The embryo-sac has eaten its way to the epidermis of apex of nucellus. Three free transitory endosperm-cells are shown.

Fig. 38. Diagrammatic drawing of ovule of *Alnus* to show course of the pollen-tube (*p.t.*). *e.s.*, embryo-sac; *f.*, funicle; *v.b.*, vascular bundle; *m.*, the closed micropyle.

Corylus Avellana.

Fig. 39. Longitudinal section of ovary of *Corylus* before the ovule is ready for fertilization. *r.*, remnant of stigmas; *p.*, perianth; *ch.*, crystals; *pa.*, loose parenchyma; *v.*, vascular bundle now become central; *cu.*, cupule.

Fig. 40. Ditto much earlier, showing stylar canal open, and vascular bundles of placentæ still not approaching the centre. *o.*, ovule; *s.c.*, stylar canal; other letters as in fig. 39.

Fig. 41. Longitudinal section of nucellus, cut June 21, 1892. *e.s.*, embryo-sac; *t.*, tapetal region; *p.*, pedicel.

Fig. 42. Upper part of similar section. *p.e.s.*, position in which a second embryo-sac lay; *a.c.*, central strand of archesporium; *r.ep.*, rudimentary epidermal cap.

Fig. 43. As before. This section shows four embryo-sacs. The elongation of tapetal cells is often seen. *p.*, pedicel; *ep.*, epidermal sac; *e.s.*, embryo-sac.

PLATE LXXI.

Corylus Avellana.

Fig. 44. Longitudinal section of ovule showing pollen-tube ascending from chalaza. *c.*, cæcum of embryo-sac; *m.*, micropyle; *p.t.*, pollen-tube.

Fig. 45. Pollen-tube and adjacent cells in the previous fig. enlarged. *n.g.*, refringent granules, ?nucleus; *c.p.*, cells of nucellus.

Figs. 46, 47. Pollen-tube abutting on cæcum of embryo-sac.

Carpinus Betulus.

- Fig. 48. Longitudinal section of nucellus (June 24th), showing area of sporogenous tissue shaded to represent the deeper-coloured granular appearance of this tissue. *s.*, spindle-shaped cells; *p.*, pedicel; *t.*, tapetum.
- Fig. 49. Longitudinal section of nucellus to show cæcum of embryo-sac. *t.*, tapetum.
- Fig. 50. Enlarged drawing from the preparation drawn in fig. 51. The egg-cell appears to have been fertilized, and has already given rise to a 3-celled embryo. The pollen-tube is becoming disorganized, and in the portion abutting upon the embryo has evidently given up a portion of its contents, though actual perforation of its apex could not be demonstrated. *em.*, embryo; *e.s.*, abortive embryo-sac; *f.e.s.*, fertile embryo-sac; *p.t.*, pollen-tube; *s.*, suspensor.

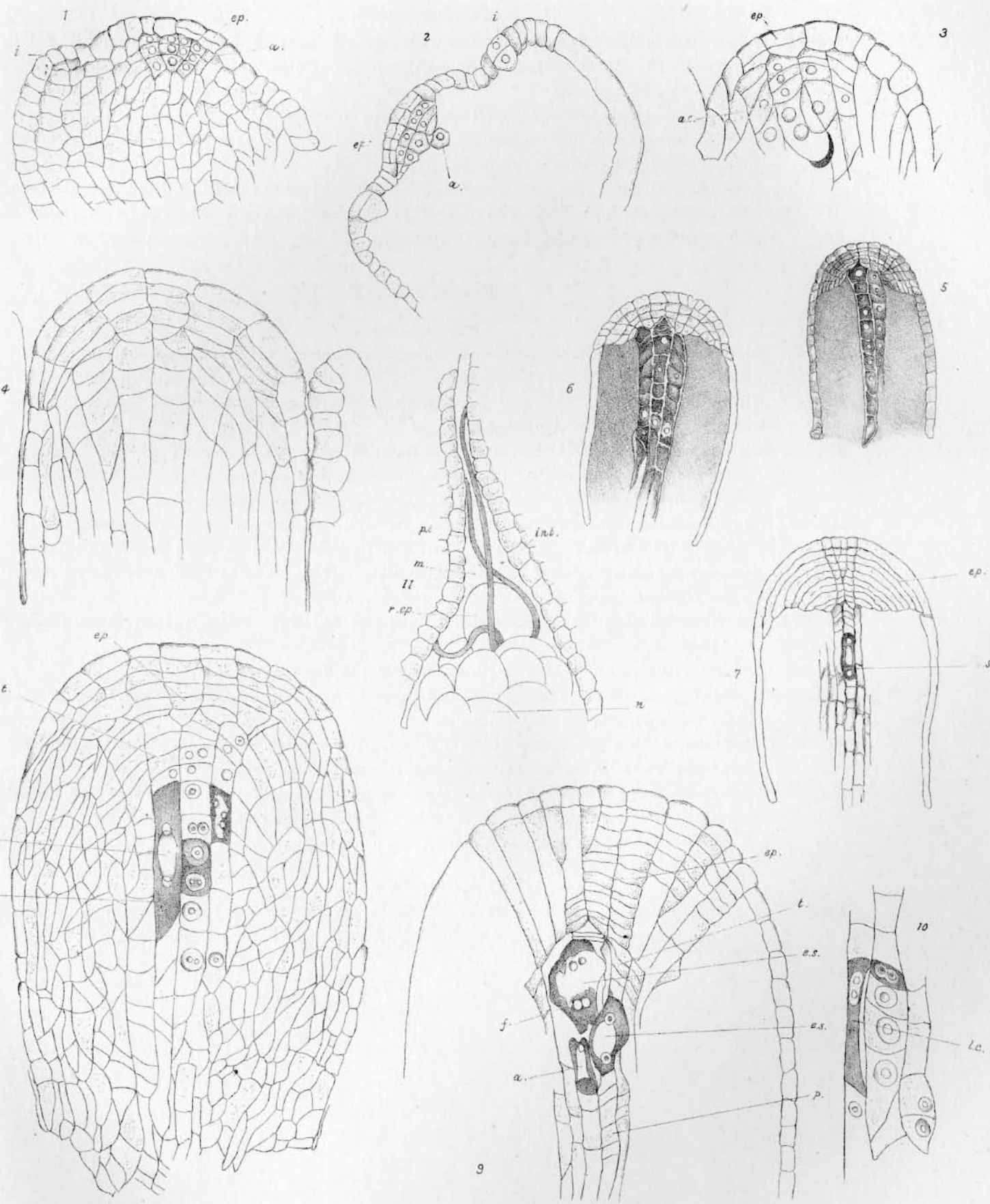
PLATE LXXII.

Carpinus Betulus.

- Fig. 51. Nucellus showing the large fertile embryo-sac with three abortive embryo-sacs (*a.e.s.*) at its micropylar extremity. Throughout the greater length of the fertile embryo-sac (*f.e.s.*) is the pollen-tube (*p.t.*), the apex of which abuts upon the small 3-celled embryo (*em.*). The chalaza is indicated by the letter *c.*
- Fig. 52. Diagrammatic longitudinal median section through ovary of *Carpinus* (equally for *Corylus*) to show course of pollen-tube. *st.*, stylar cylinder; *sc.*, sclerized tissue; *pa.*, parenchyma; *pe.*, remains of perianth; *l.*, loculus; *n.*, nucellus; *p.t.*, pollen-tube.
- Fig. 53. Longitudinal section of nucellus of *Carpinus*, dated July 1, showing four embryo-sacs.
- Fig. 54. Ditto, more truly median. Two embryo-sacs have thrown out cæca towards base of nucellus, and one remains undeveloped in the apex of nucellus. Polar nuclei in the act of fusing shown in each cæcum. *e.s.*, embryo-sac.
- Fig. 55. Base of the nucellus (shown on a smaller scale in fig. 45 *a*). *e.s.c.*, cæcum of embryo-sac; *p.t.*, pollen-tube abutting on ditto.
- Fig. 56. The whole ovule on a smaller scale, from which fig. 45 was taken.
- Figs. 57, 58. Pollen-grains of *Carpinus*, germinated in 5 % sugar-solution, after 4 days. *gr.*, pollen-grain; *a.*, enlargement at apex of tube.
- Fig. 59. Upper part of ovary of *Carpinus* (July 1), showing the position of the (?) encysted pollen-tube. *pa.*, parenchyma; *s.*, sclerized tissue; *p.e.*, encysted pollen-tube.
- Fig. 60. The encysted tube enlarged 840 diameters. *f.t.*, (?) remains of tube which was first formed from the germinating pollen-tube; *s.w.*, swollen wall after treatment with H_2SO_4 ; *l.*, lumen; *t.*, (?) tube about to pass down to funicle.

Juglans regia.

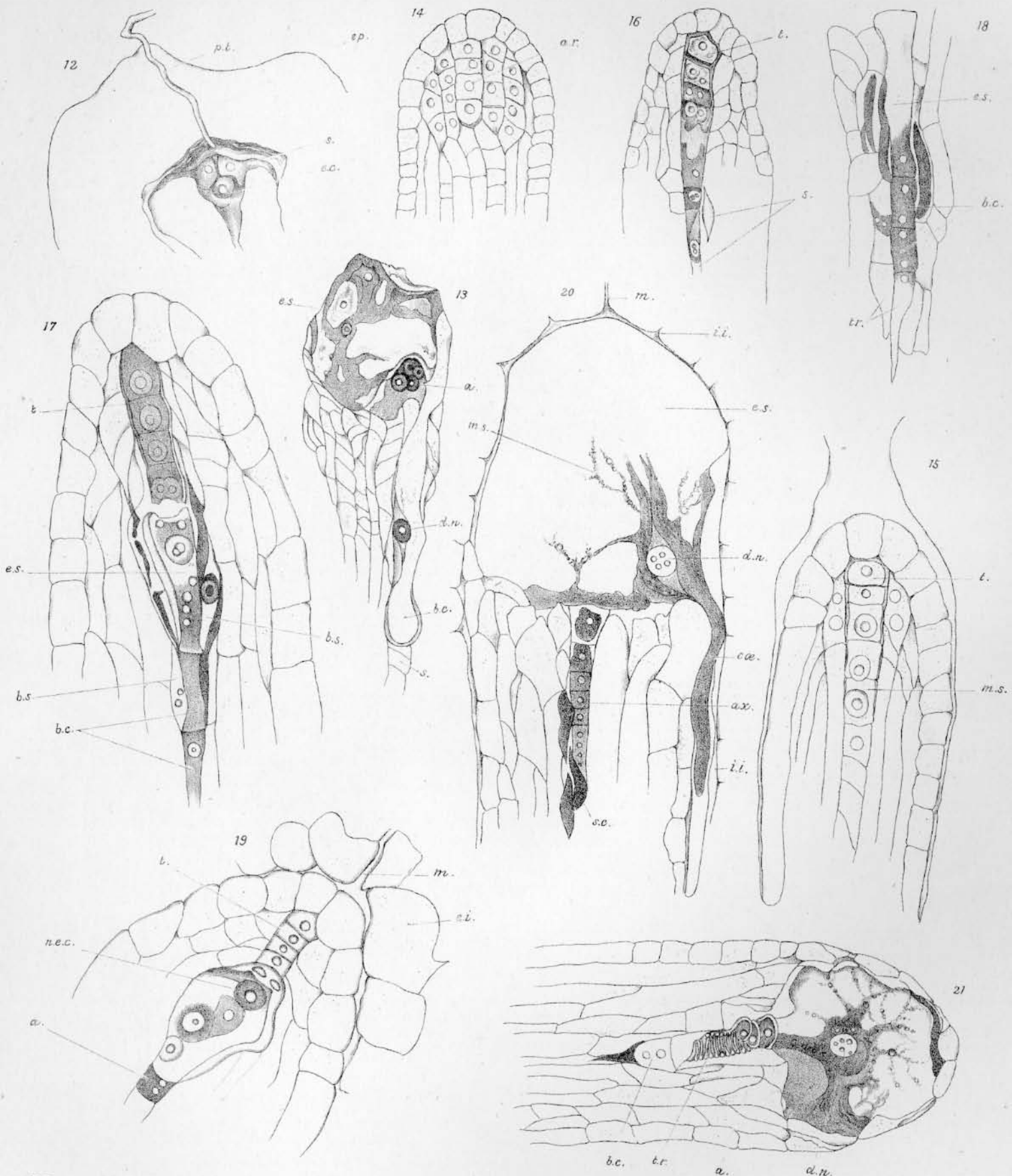
- Fig. 61. Longitudinal median section of nucellus of *Juglans*, showing the archesporial tissue. *a.c.*, central row of archesporial cells; *l.a.*, lateral row of ditto; *t.*, tapetum; *e.*, epidermis.



M. Benson del
A.R. Hammond lith.

J.N. Fitch imp.

EMBRYOLOGY OF THE AMENTIFERÆ



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